THE RELATIONSHIP BETWEEN SOCIOECONOMIC STATUS, SCHOOLS AND BONE HEALTH AMONG ADOLESCENT FEMALES IN SOUTHERN ONTARIO

Sabrina Imam, BSc. (Honours), BA.

Submitted in partial requirements of the requirements for the degree
Master of Science in Applied Health Science
(Health Sciences)

Supervisor: Dr. C. Martin Tammemägi

The Faculty of Applied Health Sciences
Brock University
St. Catharines, Ontario

ABSTRACT

We studied the association between socioeconomic status (SES), school attended and bone health measured by bone speed of sound (SOS) among adolescent females in Canada. 412 participants from six randomly selected schools in Southern Ontario were examined. Bone SOS was measured by quantitative ultrasound. Participant's school and aggregate area-based census-derived (AABCD) SES were evaluated as predictors. Mean participant age was 15.7 (SD 1.0) years. Average median family income was $68,162 (SD $19,366). Median family income was non-linearly associated with bone SOS and restricted cubic splines described the relationship. Univariate regression, accounting for clustering of participants in schools, revealed a significant non-linear association between AABCD-median family income and non-dominant tibial SOS (LRT p = 0.031). Multivariable regression revealed school to have a significant impact (LRT p = 0.0001). High schools had a strong influence on the bone health of female students and this effect overrode the effect of SES.

ACKNOWLEDGEMENTS

First, thank you to my committee as a whole for your guidance and patience. I appreciate all of your help, edits, revisions and feedback leading to an improved thesis. I learned a lot and I am profoundly grateful. Thank you all for helping me become a better researcher, writer and analyst. I would like to thank Dr. T (Dr. Martin Tammemagi) for your patience and guidance, and help in making me a better researcher and scientific writer. I would like to thank Nota (Dr. Nota Klentrou) for your help along the way and throughout this research process in improving my thesis. Thank you to Kim (Dr. Kim Gammage) for your helpful feedback on my thesis. Thank you to my external examiner Jerry (Dr. Jarold Cosby) for providing valuable feedback on my thesis. Thank you Izzy (Izabella) for being helpful and providing answers to my questions about BONES. I would also like to thank everyone involved in the BONES primary data collection. Your hard work led to a substantial amount of important data that was collected. Thank you to Mr. Ryan Waterhouse, Niagara Region Public Health for geo-coding the participants address and matching them to aggregate area-based census-derived estimators of SES from Statistics Canada 2006 Census data.

Thank you to my friends and family, including my mother, Afroz Imam, who supported me with patience along this journey in every way possible. Thank you also to my sister, Anika Imam, for your tips and support along the way. Also, thanks to those friends who have been there for me along the way.

TABLE OF CONTENTS

Page

Abstract ii

Acknowledgements..iii

Table of Contents iv

List of Tables ix

List of Figures xi

List of Abbreviations xii

CHAPTER 1: INTRODUCTION 1

1.1 Rational and purpose…………………………………………………….........3
1.2 Study
objective………………………………......………….............………......4
1.3 Hypotheses………………………………………………………………….5

CHAPTER 2: LITERATURE REVIEW 6

2.1 Bone properties……………………………………………………………6

2.1.1 Peak height velocity…………………………………………………7

2.1.2 Peak bone mass……………………………………………………...8

2.1.3 Age-related bone loss………………………………………………10

2.1.4 Bone speed of sound (SOS)……………………………………..11

2.2 Risk factors for osteoporosis and low peak bone mass..............................11

2.2.1 Non-modifiable risk factors……………………............................11

2.2.2 Modifiable risk factors……...14

2.3 Socioeconomic status...20

2.3.1 Aggregate measures of SES……………….................................21

2.3.1.1 Income…………………………………...……..................23

2.3.1.2 Education……………………….................................24

2.3.1.3 Occupation…………………...…………………………26

2.3.1.4 Family composition…………………………………...26

2.3.1.5 Housing…………...……………………………………..26

2.3.1.6 Labour force………………….………………………......27

2.3.1.7 Proportion below the low income cut-off (LICO)……….27

2.4 Population density..28

2.4.1 Niagara Region...28

2.4.2 City of Hamilton...29

2.5 Socioeconomic status and mechanisms to health outcomes.......................29

2.6 School and mechanisms to health outcomes...31

2.7 Gaps in the literature..32

CHAPTER 3: METHODOLOGY 37

3.1 BONES protocol and study design..37

3.2 Participant recruitment..38

3.3 Study sample...38

3.4 Measurements...39

3.4.1 Bone speed of sound...39

3.4.2 Outcome variables: Non-dominant tibia..41

3.4.3 Anthropometric measurements………...42

3.4.4 BONES questionnaire package……...42

3.5 Data analysis using AABCDEses..43

3.5.1 Exposure variables: Socioeconomic status....................................43

3.5.2 Residential addresses...44

3.5.3 Geo-coding and matching addresses to census areas........................44

3.5.4 Selected AABCDEses variables..45

3.5.5 School SES..46

3.6 Statistical analysis/analytic strategy...46

3.6.1 Study objective..46

3.6.2 Multilevel modeling..48

3.6.3 Multiple imputation analysis...50

3.6.4 Multivariable linear regression………...52

3.6.5 Testing assumptions of linear regression..52

Non-linear effects analysis: Restricted cubic splines....................54

3.6.6 Interaction analysis………………………..................................56

3.6.7 Multivariable logistic regression...57

CHAPTER 4: RESULTS 59

4.1 Sample characteristics...59

4.2 Pre-estimation testing for assumptions of regression modeling...............62

4.2.1 Correlation analyses...62

4.2.2 Non-linear analyses and use of restricted cubic splines...................64

4.3 Unadjusted regression...68

4.3.1 Univariate associations for distal (SES and school) with non-dominant tibial SOS (Hypotheses # 1 and 2) and proximal factors with outcome non dominant *tibial* SOS (Hypothesis #3)...............................68

4.3.2 Univariate associations between distal (SES and school) and proximal risk factors (intermediate risk factors) to non-dominant tibial SOS (Hypothesis #4)..71

4.4 Multivariable regression models..76

4.4.1 Multiple linear regression models (Hypotheses # 1 and 2)..............76

4.4.2 Examination of interactions...84

4.4.3 Post-estimation tests for assumptions for linear regression.............85

4.4.4 Multivariable logistic regression model..85

CHAPTER 5: DISCUSSION AND CONCLUSIONS 89

5.1 Pertinent study findings..89

5.2 Relation to previous research..90

5.2.1 Sample characteristics..90

5.2.2 Associations between distal factors (SES and school) and non-dominant tibial SOS (Hypotheses #1 and 2)..............................…..........91

5.2.2.1 Investigation of SES and bone health (Hypothesis #1).....96

5.2.2.2 Associations between SES and proximal factors to non-dominant tibial SOS (Hypothesis #4)..98

5.2.2.3 Associations between proximal factors and non-dominant tibial SOS (Hypothesis #3)..100

5.2.2.4 Multivariable regression models....................................101

5.2.2.5 Investigation of school and bone health (Hypothesis #2)102

5.2.2.6 Associations between school and proximal factors to non-dominant tibial SOS (Hypothesis #4)..105

5.3 Strengths and Limitations...106

5.3.1 Study limitations..106

5.3.2 Study strengths..110

5.4 Public health relevance..113

5.5 Future research directions..114

5.6 Conclusions..115

REFERENCES 117

APPENDICES 124

APPENDIX 1: Brock Osteo Nutrition Exercise Study (BONES) Questionnaire....124

APPENDIX 2: Research Ethics Board Approval for Secondary Use of Data.........156

APPENDIX 3 : Brock Osteo Nutrition Exercise Study Invitation Letter to Parent..163

APPENDIX 4 : Brock Osteo Nutrition Exercise Study Consent Form....................164

APPENDIX 5: Brock Osteo Nutrition Exercise Study Invitation Letter to School..168

List of Tables **Page**

Table 1. Descriptive characteristics of selected variables for the study sample in BONES, 2007-2008..60

Table 2. Characteristics of study students by school attended, BONES, 2007-2008......61

Table 3. Schools ranked from highest to lowest, for student SES, school SES, and non-dominant tibial SOS..62

Table 4. Unadjusted linear regression coefficients for the relationship between SES variables and non-dominant tibial bone SOS (m/s), BONES 2007-2008, adjusted for clustering (n = 368)..69

Table 5. Unadjusted linear regression coefficients for the relationship between proximal risk factors and non-dominant tibial bone SOS (m/s), BONES 2007-2008, adjusted for clustering..70

Table 6. Unadjusted linear regression coefficients for the relationship between School and non-dominant tibial SOS (m/s), BONES 2007-2008 (n = 368)..................................71

Table 7. Unadjusted linear regression coefficients, and odds ratios, for the relationships between SES (**median family income**) and the outcomes **proximal risk factors** to non-dominant tibial SOS (m/s) (intermediate factors to bone SOS), BONES 2007-2008, adjusting for clustering..72

Table 8. *t*-test results for median family income by alcohol and smoking status and family history of osteoporosis, BONES 2007-2008..73

Table 9. Unadjusted linear regression coefficients, and odds ratios, for the relationships between **School** and the outcomes **proximal risk factors** to non-dominant tibial SOS (m/s) (intermediate factors to bone SOS), BONES 2007-2008...75

Table 10a. Multivariable linear regression model for non-dominant tibial SOS (m/s), BONES 2007-2008 – Full model, adjusted for clustering (n = 374)................................77

Table 10b. Multivariable linear regression model for non-dominant tibial SOS (m/s), BONES 2007-2008 – Full model including school (fixed effects) (n = 374)....................78

Table 11. Multivariable linear regression model between proximal risk factors to non-dominant tibial SOS (intermediate risk factors) and non-dominant tibial SOS (m/s), BONES 2007-2008, adjusted for clustering (n = 378)...81

Table 12a. Multivariable linear regression model for non-dominant tibial SOS (m/s), BONES 2007-2008 - Final model, adjusted for clustering (n = 366)................................81

Table 12b. Multivariable linear regression model for non-dominant tibial SOS (m/s), BONES 2007-2008 – Final model excluding variables with p> 0.2; Model including school (fixed effects) (n = 366)..82

Table 13. Multivariable linear regression models for non-dominant tibial SOS (m/s), BONES 2007-2008 - Alternative model excluding variables with p > 0.2 and age; Model including school (fixed effects) (n = 368)..84

Table 14. Post-estimation tests of assumptions of multivariable linear regression models (assumption met vs. violated)..85

Table 15a. Multivariable logistic regression odds ratios, BONES, 2007-2008; Predicting low bone SOS vs. high bone SOS for non-dominant tibial bone– Final model, adjusted for clustering (n = 410)..86

Table 15b. Multivariable logistic regression odds ratios, BONES, 2007-2008; Predicting low bone SOS vs. high bone SOS for non-dominant tibial bone – Final model excluding variables with p > 0.2; Model including school (fixed effects) (n = 410).........................87

List of Figures **Page**

Figure 1: Bone composition (Hall, 2007)..7

Figure 2: Bone mass vs. age with optimal and suboptimal bone acquisition (Heaney et al., 2000)...9

Figure 3: Modifiable risk factors affecting bone health: Mechanism to osteoporosis.....30

Figure 4: QUS Sunlight Omnisense device...41

Figure 5: Schematic representation of possible relations between distal factors SES and school effects, proximal factors to bone SOS (i.e., alcohol use, smoking, calcium intake, physical activity, percent body fat) and bone SOS in adolescent females.........................47

Figure 6: Schematic diagram of multilevel model: Participants/students level nested in school level; investigating variance in students' bone SOS...48

Figure 7: LOWESS curve for median family income and non-dominant tibial bone SOS...64

Figure 8: LOWESS curve for calcium intake and non-dominant tibial SOS..................65

Figure 9: LOWESS curve for between % body fat and non-dominant tibial bone SOS...66

Figure 10: LOWESS curve for between BMI and non-dominant tibial bone SOS.........67

Figure 11: Testing for normality of errors for Model 2, BONES 2007-2008.................79

Figure 12: Testing for homoskedasticity for Model 2, BONES 2007-2008....................80

List of Abbreviations (units)

AABCDEses – aggregate area-based census-derived estimators of SES

BMD – bone mineral density (g/cm^3)

BMI – body mass index (kg/m^2)

BMU – basic multi-cellular unit

CI – confidence interval

DA – dissemination area

DXA – dual-energy X-ray absorptiometry

GIS – geographical information system

LICO – low-income cut-off

LRT – likelihood ratio test

OR – odds ratio

PBM – peak bone mass

PHV – peak height velocity

QUS – quantitative ultrasound

R - range

SD – standard deviation

SE – standard error

SES – socioeconomic status

SOS – speed of sound (m/s)

VIF – variance inflation factor

CHAPTER 1: INTRODUCTION

Bone and skeletal health are an integral component of physical health and well-being throughout an individual's lifespan. Strong and healthy bones are essential for an active and mobile life and lead to important benefits such as increased mobility leading to improved cardiovascular health and improved quality of life. Osteoporosis is a condition characterized by low bone mass and increased susceptibility to fractures (Dominguez, Scalisi, & Barbagallo, 2010). It is often considered a disease of old age, as individuals are more likely to experience osteoporosis as they age (i.e. women over age 50, men over age 60) (Osteoporosis Canada, 2010). With the large aging population, it is increasingly becoming a major and global public health concern (Dominguez, et al., 2010). Nearly 2 million Canadians (Osteoporosis Canada, 2010), and 44 million Americans (National Institute of Arthritis and Musculoskeletal and Skin Diseases, 2011), are living with osteoporosis. Women in particular are at risk for developing osteoporosis, with 1 in 4 women over the age of 50 reported to have osteoporosis in Canada (Osteoporosis Canada, 2010). At least 1 in 3 women will suffer from an osteoporotic fracture during their lifetime compared to 1 in 5 men (Osteoporosis Canada, 2010). Currently in Canada, treating osteoporosis and the fractures it causes costs the health care system an estimated $1.9 billion annually, and osteoporotic hip fractures consume more hospital bed days than stroke, diabetes, or heart attack (Osteoporosis Canada, 2010). In fact, the lifetime risk of sustaining a hip fracture is 1 in 6 which is greater than the lifetime risk of developing breast cancer (1 in 9) (Cummings, Black, & Rubin, 1989). At least 80% of fractures in people aged 60 and older are related to osteoporosis. Annually, osteoporosis causes 70 to 90% of the 30,000 hip fractures that occur. Mortality is a risk since up to 30% of these osteoporosis-related hip fracture cases result in death, with 23% of those who sustain a hip fracture dying within a year (Osteoporosis Canada, 2010). These grave consequences of this serious condition further demonstrate the need for prioritizing health promotion, prevention strategies and research in this area. Although osteoporosis is most prevalent in older adults, osteoporosis can strike at any age and is often referred to as a geriatric condition with pediatric roots (Osteoporosis Canada, 2010). Much of an individual's peak bone mass (PBM) is acquired during the critical growth period during early adolescence (Novotny et al., 2004) and accrued by approximately age twenty in females. Peak bone mass can be defined as the amount of bony tissue present at the end of skeletal maturation (Bonjour, Theintz, Law, Slosman, & Rizzoli, 1994). After PBM is attained, natural bone loss due to aging soon begins. Greater understanding of PBM accrual should be a research and public health priority particularly for investigating bone health among young females. It is important for children and adolescents to build strong and healthy bones since PBM is attained by an individual's late teens to twenties, and building strong bones early in life can be the best defence against osteoporosis later in life (Osteoporosis Canada, 2010). Aside from genetics, risk factors associated with osteoporosis and poor bone health include low calcium intake, smoking, alcohol consumption, too little exercise, low weight and certain medications and steroids (Webb, 2006).

Socioeconomic status (SES) may modify these risk factors but the effect of SES on bone properties among young females is unclear and requires further investigation. SES is a very important predictor of health and health behaviours (Public Health Agency of Canada, 2012). It can be measured at the individual level, often by education, occupation or income. It can also be measured by the characteristics of the community one lives in. The latter method often uses aggregate area-based census-derived estimators of SES (AABCDEses), such as median household income. The latter approach is of great importance in medical research because it can be obtained from a patient's home address, even when individual level SES data are absent or of poor quality. AABCDEses have been shown to be a valuable predictor of health outcomes, such as cancer survivorship (Brenner, 2008).

1.1 Rationale and purpose

With the growing aging population, and thereby the increasing incidence and prevalence of osteoporosis, the burden on the health care system is increasing. It is imperative that health promotion and prevention measures are undertaken with greater urgency, particularly for young females. Prevention should begin in childhood and adolescence when the precursor for osteoporosis, lack of adequate peak bone mass, first begins to develop. Early prevention strategies are believed to offer lifetime benefits and be more effective than late interventions. Thus, it is imperative that research is conducted into female bone health in early adolescence.

Investigating SES as a risk factor is imperative in order to understand and identify at-risk populations. Southern Ontario exhibits a range of SES including 12% of children below the low-income cut-off (Willms, 2010). As well, since considerable time periods of children and adolescents' lives are spent in school, schools may be useful for promoting positive health behaviours and may play a critical role in the bone health of adolescent females. Examining the relationship between SES, school, and bone health in adolescent females will help elucidate the mechanisms of social determinants of bone health, identify those at greater risk for osteoporosis and may assist in developing health promotion strategies towards preventing osteoporosis.

The purpose of this study was to develop a better understanding of the relationship between SES, schools and bone health using AABCDEses from Statistics Canada Census 2006. More specifically, the objective was to examine the effect of AABCDEses on bone properties as measured by trans-axial Quantitative Ultrasound (QUS). QUS measures the bone speed of sound (SOS) and is increasingly becoming an important measure of bone properties because of its ability to capture multiple properties of bone such as bone micro-architecture, cortical thickness and geometry in addition to bone mineral density (Omar, 2006). By investigating the relationship between SES, school, and bone SOS, new insights can be revealed into the factors influencing bone properties among adolescent females. The current study will expand on current knowledge and examine whether SES and school are associated with bone SOS among adolescent females in Southern Ontario, specifically the Niagara region and the City of Hamilton.

1.2 Study objective

The objective of this study was to investigate the association between SES, specifically median family income, school attended and bone SOS of the non-dominant tibia in adolescent females in Southern Ontario.

1.3 Hypotheses

The following hypotheses were evaluated:

1) Socioeconomic status and non-dominant tibial bone speed of sound are positively associated among a Canadian adolescent female population.. This hypothesis was based on previous literature which found that SES was significantly positively associated with bone mineral density in adult populations (Brennan S. L, 2009; Brennan et al., 2010).
2) School attended is associated with non-dominant tibial bone speed of sound among a Canadian adolescent female population. This hypothesis is supported by a small research study in Turkey investigating two primary schools and bone SOS which suggested an association, however with a small sample size (Akarirmak, 1996).
3) Proximal factors to non-dominant tibial speed of sound, such as age, percent body fat, calcium intake, physical activity, family history of osteoporosis, regular smoking and alcohol consumption are associated with non-dominant tibial speed of sound.
4) Socioeconomic status and school are associated with proximal factors related to non-dominant tibial speed of sound, such as percent body fat, regular smoking, alcohol consumption, physical activity, calcium intake and family history of osteoporosis, among a Canadian adolescent female population.

CHAPTER 2: LITERATURE REVIEW

This chapter will provide an overview into bone properties, peak bone mass and osteoporosis. Risk factors associated with low peak bone mass and osteoporosis will be described to provide insight into this condition. Finally, the core dimensions of SES will be described. This chapter ends with a description of the gaps in the literature and how the current study addresses those gaps.

2.1 Bone properties

Bones are living, dense connective tissues that make up the skeleton in the body, and there are 206 bones in an adult human skeleton (Osteoporosis Canada, 2010). Bones function to store mineral reserves such as calcium and phosphorus, thereby keeping bones strong, and releasing these minerals into the body when needed (National Institute of Arthritis and Musculoskeletal and Skin Diseases, 2011). Bone is continually being renewed and replacing old and micro-damaged bone, through a process called bone remodelling, by way of cells constituting bone called *osteoblasts* and *osteoclasts* (Seeman, 2003). Osteoblasts contribute to bone formation, while osteoclasts are responsible for bone resorption, reducing bone volume (Clarke, 2008).

Bone is composed of two types of bone tissue, cortical and trabecular. Cortical or compact bone tissue make-up the hard outer layer of bone and have minimal gaps with a porosity of 5-30%. It accounts for 80% of the total bone mass of an adult skeleton (Clarke, 2008). Trabecular bone tissue fills the interior of bone and is also called cancellous or spongy bone. It is composed of a network of plate and rod-like elements allowing space for blood vessels and marrow (Clarke, 2008), as illustrated in **Figure 1**.

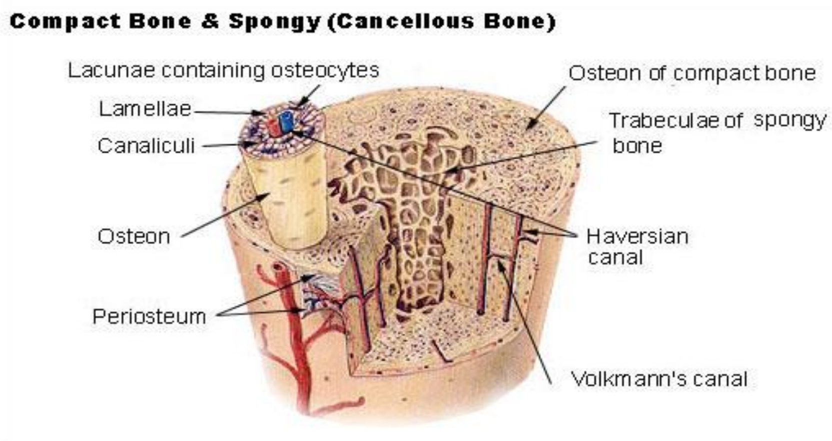


Figure 1: Bone composition (Hall, 2007)

Bone strength is a result of bone mineral density and bone geometry. Cortical thickness, elasticity and micro-architecture of the bone all also contribute to bone quality (Omar, 2006). Bone mineral density is a common indicator of bone strength and is defined as grams of bone mass per cubic centimetre.

2.1.1 Peak height velocity

Peak height velocity (PHV) is the point in pubescence where the maximum rate of growth occurs (Little, Song, Katz, & Herring, 2000). The age at which PHV occurs for girls is 9 to 12 years. Bone mineral density remains constant or increases slightly during growth as bone mass accrual occurs in proportion to the enlarging whole bone. As described by Seeman (2003), “Greater strength of long bones in men vs. women is the result of differences in size and geometry of the bone and not density. Growth builds a bone that is bigger, not a more dense bone”. Cooper et al (1995) suggested that growth primarily determines the size of the skeletal envelope, with its trajectory established by age 1, while the mineral density within the skeletal envelope is modulated by factors such as physical activity, and following the end of linear growth, may contribute to the consolidation of bone. The mineralized skeleton is composed of the periosteal, or outer surface, and endosteal, or inner surface (Seeman, 2003). There are differences in bone width between girls and boys that are established during the peri-pubertal period attributed to differences in periosteal bone formation and endocortical apposition (growth of layers) (Seeman, 2003). Estrogens are what stimulate endosteal apposition in females, and inhibit periosteal apposition resulting in a narrower bone in girls than in boys (Seeman, 2003). Men can tolerate a larger load on bone due to a larger skeleton than women, and since men are generally taller and heavier the absolute load imposed on the body is greater in young men than young women (Seeman, 2003).

2.1.2 Peak bone mass

Peak bone mass (PBM) can be defined as the amount of bony tissue present at the end of skeletal maturation (Bonjour, et al., 1994). The majority of an individual's PBM is acquired during the critical growth period during early adolescence (Novotny, et al., 2004) and according to some studies, accrued up to age 25 to 30 (Humail, 2009). Osteoporosis Canada has suggested that PBM is accrued by age 16 in females and age 20 in males, and studies have indicated that 90% of optimal PBM is acquired by age 18 years (Murphy, Ni Dhuinn, Browne, & Orathaille, 2006). Once PBM is acquired, bones will be at their strongest. After the point that PBM is acquired, natural bone loss due to aging begins, approximately after the age of thirty. It is imperative that children and adolescents build strong bones to their genetically pre-determined PBM potential since PBM is attained by an individual's late teens to late twenties, and building strong bones early in life can be the best defence against osteoporosis later in life (Osteoporosis

Canada, 2010). Adolescent females need to take advantage of the window of opportunity in peri-puberty to develop healthy bones early in life in order to maintain lifelong healthy bones and prevent osteoporosis later in life. PBM is influenced by a number of factors including race, body size, amount of exercise, smoking, type of diet, calcium intake, exposure to sunlight and vitamin D intake and hormonal activity (National Institute of Arthritis and Musculoskeletal and Skin Diseases, 2011).

Low PBM essentially predisposes individuals to future risk of osteoporosis and thus health promotion strategies towards optimizing a healthy peak bone mass during the adolescent years are critical. As shown in **Figure 2**, failure to achieve optimal bone mass at the end of adolescence leaves an individual with much less reserve to withstand the normal losses of bone during later life (Heaney et al., 2000).

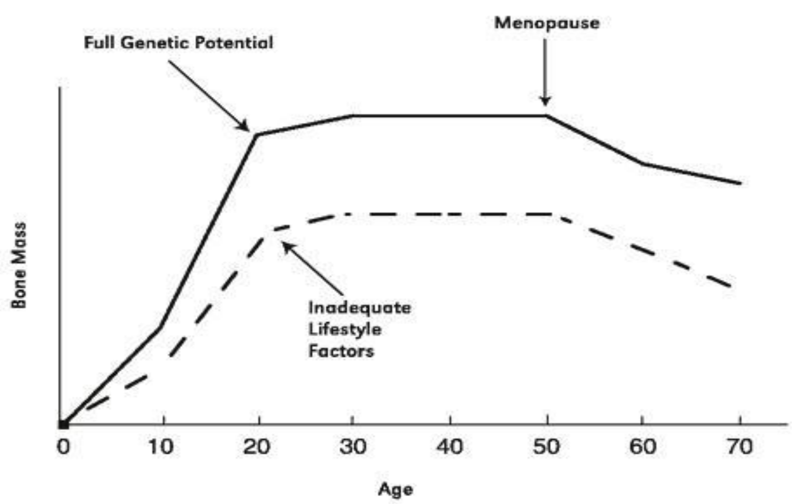


Figure 2: Bone mass vs. age with optimal and suboptimal bone acquisition (Heaney, et al., 2000)

2.1.3 Age-related bone loss

After PBM is acquired, bone size loss does not occur until approximately the mid-thirties (Rizzoli, Bonjour, & Ferrari, 2001). For net bone loss to occur the volume of bone resorbed must be greater than the volume of bone formed (Seeman, 2003). Osteoclasts resorb bone leaving a cavity on the trabecular and endocortical surfaces within the cortex, and thereafter osteblasts fill the cavity with new bone that mineralizes (Seeman, 2003). The balance becomes negative beginning after the age of thirty most likely due to an early reduction in bone formation and not due to an increase in resorptive removal of bone. Once bone resorption is greater than bone formation, bone loss begins and bone cortex and trabeculae become thinner and perforated resulting in increased cortical porosity and a destruction of trabeculae, which account for age-dependent bone loss (Rizzoli, et al., 2001).

Since bone mass is lost gradually throughout life (Mosekilde, Ebbesen, Tornvig, & Thomsen, 2000) when more bone is removed than formed resulting in net bone loss, which usually begins in the mid-30-40's, it is essential that optimal PBM be acquired to defend against osteoporosis. Bone loss begins slowly and then particularly accelerates after menopause in women when the body no longer produces estrogens (Clarke, 2008). The net bone loss from the whole bone is greater in women than in men (Seeman, 2003). Absence of estrogens play an important role in the pathogenesis of bone fragility during growth and aging as will be discussed further in the section reviewing modifiable risk factors.

2.1.4 Bone speed of sound (SOS)

The speed of sound through bone measured by ultrasound instrument is increasingly being utilized to assess the quality and strength of bone, and was used to collect bone health data as part of this study. Speed of sound travels faster through non-porous solids and liquids than through air, therefore greater bone mass will result in a faster speed of sound value. In bone, the speed of sound waves propagate along the cortical bone measured in metres/second (Foldes, Arnon, & Popovtzer, 1996). Bone SOS captures information about cortical thickness, elasticity and micro-architecture of bone, as well as bone mineral density, all contributing to bone quality (Omar, 2006). Thus, SOS provides an overall summary statistic measuring bone health. Bone SOS is measured by transaxial quantitative ultrasound (QUS).

2.2 Risk factors for osteoporosis and low peak bone mass

Many risk factors are known to be associated with osteoporosis, and also are known to be inversely associated with PBM during growth (Rizzoli, et al., 2001). In addition to these factors being associated with PBM acquisition, they are also associated with the maintenance of bone mass during adulthood as well as bone loss later in life (Rizzoli, et al., 2001). These factors fall into two categories, non-modifiable and modifiable risk factors, and are described below.

2.2.1 Non-modifiable risk factors

There are several risk factors for low peak bone mass and osteoporosis which are non-modifiable including heredity, age, race, and gender.

Genetic predisposition and hereditary factors play a prominent role in developing osteoporosis (Rizzoli, et al., 2001). Individuals with a family history of osteoporosis have an increased risk for osteoporosis (National Institute of Arthritis, 2008), and research indicates that those having a parent who had a hip fracture are at greater risk of developing osteoporosis (Osteoporosis Canada, 2010). According to Rizzoli et al. (2001), a major proportion of variance in bone mineral density values (BMD) for both genders is due to genetic factors which are expressed from before puberty and until PBM is achieved. Heritability between parent and offspring for BMD has been estimated in the range of 60% (Rizzoli, et al., 2001); therefore genetics accounts for approximately 60% of the variance in BMD.

Race and ethnicity can be a risk factor for osteoporosis for women. Research indicates that White and Asian women are most likely to develop osteoporosis. Also at risk, but less so than White or Asian women, are Hispanic and African American women (National Institute of Arthritis, 2008).

Gender also influences the risk of osteoporosis, as women are more likely to develop osteoporosis than men (Osteoporosis Canada, 2010). In regards to PBM, women tend to have lower PBM than men. Before puberty boys and girls acquire bone mass at similar rates, however after puberty, men acquire greater bone mass than women (National Institute of Arthritis and Musculoskeletal and Skin Diseases, 2011). Men also have a longer period of bone mass gain therefore acquiring greater bone size and cortical thickness (Rizzoli, et al., 2001). Currently 1 in 4 women over the age of 50 are reported to have osteoporosis in Canada, in contrast to the 1 in 8 men over the age of 50 in Canada

(Osteoporosis Canada, 2010). Recent reports suggest that men are also vulnerable to osteoporosis, however women still lose bone at a greater rate than men at 2 to 5% per year as they approach menopause (Osteoporosis Canada, 2010), compared to men who lose about 0.5% bone mass per year after age 50 (Knoke & Barrett-Connor, 2003).

The risk for osteoporosis also increases with increasing age (National Institute of Arthritis, 2008). Age increases the risk of developing osteoporosis and fractures, with particularly greater risk for those aged 65 years or older (Osteoporosis Canada, 2010). Age-related bone loss accelerates after menopause in women (Rizzoli, et al., 2001), increasing the risk for osteoporosis. In fact, there is a decrease in trabecular bone density of approximately 50% during normal aging (Mosekilde, et al., 2000). Therefore the incidence of osteoporotic fractures is expected to rise as the proportion of older populations increase worldwide (Dominguez, et al., 2010).

Estrogens are hormones found in the body which cease to be produced during menopause in women. Among young females, estrogen can have an effect on PBM such that girls who had their first menstrual cycle at an early age, or use oral contraceptives which contain estrogen, tend to have a higher bone mineral density (National Institute of Arthritis and Musculoskeletal and Skin Diseases, 2011). As estrogen deficiency has been linked to a greater risk for osteoporosis (Rizzoli, et al., 2001), on the other end of the age spectrum, women who enter menopause earlier are exposed to less estrogen therefore are at greater risk for osteoporosis than those who enter menopause at a later age. Estrogen deficiency can accelerate bone loss by indirectly accelerating bone turnover and by uncoupling bone formation from resorption (Rizzoli, et al., 2001). The rate of bone remodelling increases with estrogen withdrawal due to more bone being resorbed than replaced thus producing a net negative basic multicellular unit (BMU) balance (Seeman, 2003). A net negative BMU is the basis of bone loss, and many net negative BMU`s increase the porosity of the bone resulting in a fall in BMD (Seeman, 2003). Estrogen deficiency also contributes to osteoporosis and bone loss by increasing the life span of osteoclasts and decreasing the life span of osteoblasts, thereby increasing the porosity of the bone. The porosity of bone is also increased (Seeman, 2003) through an increase in the protein interleukin-1, which is usually blocked by estrogen.

2.2.2 Modifiable risk factors

Modifiable risk factors are factors that can be controlled and include physical activity, nutrient intake (particularly calcium and vitamin D), body weight, regular alcohol use and cigarette smoking. Risk factors for osteoporosis are additive such that there is a greater risk of developing osteoporosis with multiple risk factors (Osteoporosis Canada, 2010). The following factors are associated with risk of suboptimal PBM and developing osteoporosis.

Physical activity is one of the major modulators of bone health (Lanou, Berkow, & Barnard, 2005). Weight-bearing exercises such as walking, running, and weight-lifting have been suggested as key physical activities for improving bone mass (Novotny, et al., 2004), as well as impact training such as jumping and aerobics (Osteoporosis Canada, 2013). Impact training leads to bone formation and affects bone by creating small deformations in the bone from the exerted force of impact exercise, which then signals osteoblasts to migrate to the surface of the bone (Baechle, 2008). The osteoblasts then secrete the protein collagen which fills the gaps created by the deformations, and the collagen proteins then mineralize, increasing the density of the bone matrix (Baechle, 2008). Childhood weight-bearing physical activity has been recognized as an important determinant of PBM, and thus a possible prevention tool for osteoporosis (MacKelvie, Khan, Petit, Janssen, & McKay, 2003). Strength training or resistance training, which involves high muscle and joint compressive forces, has also been suggested to have a positive influence on bone mass (Blimkie et al., 1996). Bone density was found to be positively correlated with muscular strength in adolescent males and females who competed in competitive weight lifting (Blimkie, et al., 1996). Research suggests that physical activity levels during ages 12 to 18 years exert a greater influence on adult bone mineral density than does calcium intake during these ages (Lanou, et al., 2005). Since physical activity in youth promotes the achievement of optimal PBM, it also greatly influences lifelong skeletal adequacy (Murphy, et al., 2006). A sedentary lifestyle puts an individual at greater risk of fractures during stresses such as lifting or bending down (Webb, 2006). Evidence indicates that physical activity declines in adolescence, worldwide, and especially among girls (Murphy, et al., 2006). This further demonstrates the need for health promotion towards physical activity among this population.

Body weight has been documented to be a strong predictor of bone mineral content in children (Du et al., 2002; Moro et al., 1996). Research suggests that a smaller frame is a risk factor for osteoporosis. Weight of less than 57 kg (126 lbs) is considered a small frame. Also, weight loss greater than 10% of a person's weight at age 25 is a risk factor (Webb, 2006). Regarding obesity and osteoporosis, although previous epidemiological studies had suggested that high body weight or body mass index (BMI) was linked to high bone mass and that weight loss may cause bone loss, new research suggests that the important variable is actually percentage of body fat, which takes into account the mechanical loading effect of body weight (Zhao et al., 2007). This suggests that although a greater body weight and obesity may seem to be correlated with increased bone mass, that in fact once mechanical loading of total body weight on bone mass was controlled for, increasing fat mass may not have a beneficial effect on bone mass (Zhao, et al., 2007). Increasing percent of fat mass may be linked with diminishing bone density, and losing weight and fat mass would likely contribute to greater bone density (Zhao, et al., 2007). A Canadian study that investigated 60 females between 10 and 19 years of age also found that body fat was negatively associated with bone mineral content (Weiler et al., 2000). A healthy body fat composition for an adolescent female is considered to fall between 22 to 25% (Roitman & Herridge, 2001). With these research findings in mind, strategies for obesity prevention would also benefit bone health.

Calcium intake has been well established as contributing to bone integrity (Lanou, et al., 2005) and bone metabolism and health are positively impacted by dietary calcium intake (Cashman, 2002). Calcium is the most abundant mineral in the human body and over 99% of the body's supply of calcium is found in bones and teeth, with the skeleton serving as a reservoir for calcium (Cashman, 2002). The remaining 1% of the body's supply of calcium is found in blood, extracellular fluid, muscle and other tissues, playing vital roles in muscle contraction and nerve transmission (Cashman, 2002). The concentration of ionised calcium in the plasma is maintained by the homeostatic control of calcium in bone by processes such as absorption, excretion and storage (Cashman, 2002). Dietary calcium is primarily needed for bone mineral deposition (Greer & Krebs, 2006). Eighty to ninety percent of bone mineral content is composed of calcium and phosphorus, with the remaining composition including protein (Ilich & Kerstetter, 2000). Calcium and phosphate combine together to contribute to the crystalline complex hydroxyapatite which provides the solid and rigid structure of bone (Rey, Combes, Drouet, & Glimcher, 2009). During normal growth and development of the skeleton, calcium accumulates in the skeleton at approximately 150 mg per day until maturity when the skeleton is in calcium equilibrium (Cashman, 2002). Increased calcium intake has been found to be associated with a higher bone mass accrual rate (measured by BMD), in children and adolescents, by approximately 1 to 5% depending on the skeletal site (Cashman, 2002).

Calcium is most commonly contained in foods, such as milk products, soybeans, fortified orange juice, whole wheat bread, broccoli, oranges, bok choy, dried figs, and in some fish such as salmon (Osteoporosis Canada, 2010). It is important that adequate dietary calcium be consumed from early life for PBM to be reached and so that skeletal mass can be maintained and age-related bone loss minimized (Cashman, 2002). Chronic calcium deficiency has been linked to reduced bone mass and osteoporosis (Cashman, 2002). It is likely that PBM is negatively impacted by low calcium intake (Cashman, 2002). Daily calcium recommendations for females aged 9 to 18 years old range from 800 – 1300 mg (Osteoporosis Canada, 2010). Daily calcium intake recommendations for females aged 11 to 24 years range from 1200 - 1500 mg per day. Consumption of foods containing excess sodium, as well as caffeine, should be consumed with caution as reports have suggested that calcium loss through the urine is increased by excess salt (sodium) consumption and caffeine intake (Osteoporosis Canada, 2010).

Vitamin D is essential in maintaining a healthy mineralized skeleton in humans (Holick, 1996). Of particular importance is vitamin D3, which is endogenously synthesized in the skin by photo-production under the influence of sunlight and ultraviolet radiation (Holick, 1996; Lips et al., 2010). Vitamin D3 is then metabolized in the liver and kidney to form 1,25-dihydroxyvitamin D (1,25(OH2)D (Holick, 1996). The major biological function of 1,25(OH2)D is to maintain essential cellular functions and to promote mineralization of the skeleton by keeping the serum calcium and phosphorus concentrations within the normal range (Holick, 1996). Vitamin D helps maintain blood calcium in the normal range by 1,25(OH2)D, increasing the efficiency of intestinal calcium absorption (Darwish & DeLuca, 1993; Holick, 1996). Since vitamin D signals the intestines to absorb calcium, low levels of vitamin D lead to the body breaking down bone to release calcium from bone to get the calcium it needs, lowering bone mineral density, and increasing the risk of fractures. Deficiency in vitamin D can result in mineralization defects, resulting in conditions such as rickets and osteomalacia, as well as secondary hyperparathyroidism which results in high turnover bone resorption, and consequently osteoporosis and fractures (Lips, et al., 2010). Vitamin D insufficiency can also decrease muscle strength and balance. A 3-year prospective study on peri-pubertal Finnish girls found that girls with hypo-vitaminosis D had a 4% lower bone mineral density accumulation from baseline than did girls with normal vitamin D status (Lehtonen-Veromaa, Mottonen, Nuotio, Irjala, & Viikari, 2002) further illustrating the importance of vitamin D intake, either through vitamin D supplementation or from sunlight, among young females. The daily recommendation of vitamin D for females aged 4 to 50 years is 400 IU (Lips, et al., 2010), although recent reports recommend a daily intake of up to 1000 IU (Osteoporosis Canada, 2010). Although vitamin D is generally uncommonly found in dietary foods (Holick, 1996), some food sources are egg yolks, salt water fish and liver (National Institute of Arthritis and Musculoskeletal and Skin Diseases, 2011). Most humans meet their vitamin D requirement through exposure to sunlight (Holick, 1996), however individuals with minimal sun exposure may need to follow a recommended dietary intake of vitamin D or vitamin D supplementation (Health Canada, 2011).

Tobacco use increases the risk of fractures in old age and is dose-dependent (National Institute of Arthritis and Musculoskeletal and Skin Diseases, 2011). Increased intensity and duration of cigarette smoking is associated with this risk (National Institute of Arthritis and Musculoskeletal and Skin Diseases, 2011). Second-hand smoke during youth and early adulthood is associated with an increase in the risk of developing low bone mass (National Institute of Arthritis and Musculoskeletal and Skin Diseases, 2011). Smoking also leads to decreased estrogen production. Women who smoke tend to experience menopause earlier, which may lead to increased bone loss, since estrogen production stops during menopause. Although quitting smoking appears to reduce the risk of low bone mass and fractures, it may take several years to lower a former smoker’s risk of fractures (National Institute of Arthritis and Musculoskeletal and Skin Diseases, 2011). Thus the importance of excluding smoking as a life style behaviour is further demonstrated since in addition to other health concerns related to smoking, bone health is also at risk.

Regular excess consumption of alcohol (consistently more than 2 drinks a day) elevates the risk of osteoporosis and fractures (Osteoporosis Canada, 2010). Chronic alcohol use interferes with the balance of calcium in the body and also affects the production of hormones that have a protective effect on bone. Excess alcohol use also affects vitamin D production necessary for calcium absorption (National Institute of Arthritis and Musculoskeletal and Skin Diseases, 2011). Research also suggests that chronic heavy drinking particularly during adolescence and young adulthood may dramatically compromise bone quality as well as increase the risk for osteoporosis later in life (Sampson, 2002). In addition to the aforementioned research, it has been suggested that even if heavy alcohol use is terminated, the effect of heavy alcohol use on bone cannot be reversed (Sampson, 2002). Therefore alcohol should not be excessively consumed throughout early adolescence and adulthood.

Steroid and corticosteroid medications are anti-inflammatory drugs that decrease swelling and activity of the immune system (van Staa, Leufkens, & Cooper, 2002). The use of corticosteroids has been found to reduce bone formation and inhibit osteoblast replenishment thereby decreasing bone formation and increasing the risk of fracture. This heightened risk is dose-dependent (van Staa, et al., 2002).

2.3 Socioeconomic status

Socioeconomic status describes the position of a person in society, based on a combination of occupational, economic, and educational criteria (Canadian Population Health Initiative Council, 2008), as well as power and prestige. Socioeconomic status is estimated by three core markers: income, education and occupation. Housing characteristics, and family characteristics, such as two-parent versus lone parent families, are also traditional measures of socioeconomic status (Dall, 2006). Socioeconomic inequalities can lead to exposures such as hazardous occupations, poor diet, and limited access to health care (Kogevinas, 1997). Higher income is associated with greater spending power, better housing, diet, and medical care. Occupation measures prestige, responsibility, physical activity and work exposures. Education facilitates skills required for acquiring positive social, psychological, and economic resources (Winkleby, Jatulis, Frank, & Fortmann, 1992). Socioeconomic status has been considered one of the strongest and most consistent predictors of a person's morbidity and mortality experience (Winkleby, et al., 1992). According to the Public Health Agency of Canada (2012), socioeconomic status is the number one determinant of health status, more important than genetic endowment. The key determinants of health in order of importance according to the Public Health Agency of Canada are: 1. Income and social status, 2. Social support networks, 3. Education and literacy, 4. Employment/working conditions, 5. Social environments, 6. Physical environments, 7. Personal health practices and coping skills, 8. Healthy child development, 9. Biology and genetic endowment, 10. Health services, 11. Gender, and 12. Culture (Public Health Agency of Canada, 2012). Thus, examining the impact of SES on bone health status among Canadian adolescent females is important.

2.3.1 Aggregate measures of SES

Aggregate area-based census-derived estimators of SES (AABCDEses) will be the measure of SES utilized in this study. AABCDEses measures are summary measurements of the SES of the community an individual lives in, such as median household income or the proportion below the low-income cut-off. The size of the areas that the estimators are based on usually vary from as small as 400 to 700 people (dissemination area; DA), to larger areas consisting of 2,500 to 8000 people (census tract; CT) (Statistics Canada, 2011). For this study, the dissemination area was used, and will be discussed further in Chapter 3 of this dissertation.

SES can be measured at the individual or aggregate level, but research in the past decade has reinforced the theory that aggregate measures of health status form one of the basic tools of population health research. Aggregate SES variables are becoming increasingly important in epidemiologic and public health research (Kaufman, Cooper, & McGee, 1997). According to the 2006 Socioeconomic Atlas, analysis of individual level SES may not adequately capture important predictors of health (Dall, 2006). A combination of variables often best reflects the status of a population since there are a number of variables which contribute to any particular dimension of SES (Dall, 2006). As stated in the 2006 Socioeconomic Atlas, "Research in the past decade indicate that analysis of individuals alone may not adequately capture factors associated with health status, and aggregate measures are a basic tool of population health research" (Dall, 2006).

The use of aggregate SES variables can be easier to collect than individual data. As well, individual level SES measures such as income are often self-reported and underreported, and inaccurately estimated. Whenever individual-level SES indicators are absent from available records, but the addresses of individuals are known, SES can be analyzed by assigning to individuals the SES value of the community (for example, by postal code or census tract) (Kaufman, et al., 1997). However, there are potential issues and biases associated with the use of aggregated SES measures, such as misclassification bias (Kaufman, et al., 1997). *Misclassification bias* occurs when subjects are misclassified due to inaccuracies in data collection methods, thereby misclassifying a subject's exposure status (Gordis, 2009). For example, in this study, the participant's address could be on the boundary between two dissemination areas and therefore potentially misclassified into the unrepresentative dissemination area thus misclassifying her level of SES. It is also possible that the DA boundaries are not accurate in reflecting that parcel of land. An advantage of aggregated SES measures is that group effects will be encoded in the aggregated SES variables that are independent of the individual effects (Kaufman, et al., 1997). This can be viewed as an advantage, since capturing information at a group level can provide insights not previously considered.

2.3.1.1 Income

Although this study investigated aggregate level SES, an individual's income can reflect his or her financial earnings, but also spending power, housing, diet, and medical care (Winkleby, et al., 1992). Therefore income also measures material disadvantage. According to the Socio-economic Indicators Atlas (2006), lower income has consistently resulted in lower health outcomes in measures such as morbidity and mortality. Lower income tends to result in a lack of resources, less access to nutritious foods, inadequate housing, residing in unsafe neighbourhoods, working in more hazardous conditions, and increased exposure to stress (Public Health Agency of Canada, 2012). This can also lead to poorer health-related behaviours such as regular smoking and over-consumption of alcohol (Public Health Agency of Canada, 2012). The two main measures of AABCDEses for income used by Statistics Canada are median and average household income. Median and average household income of a community can reflect the affluence of the community where the person lives. Median and average family incomes are important AABCDEses. Family income refers to the income of the economic family. The economic family defined by Statistics Canada refers to a group of two or more persons who live in the same dwelling and are related to each other by blood, marriage, common-law or adoption (Statistics Canada, 2011). According to a report by the Government of Ontario, 1 in 6, that is 17%, of Canadian children aged 0 – 5 years, and 15% of Ontario children, are living in families with annual family incomes below $30,000 (Willms, 2010). According to Statistics Canada, 9.4% of Canadians are in a family whose income is below the low-income after-tax income cut-off, as of 2008. The low-income cut-off for four-person families in medium-sized cities was $21,359 as reported by Statistics Canada (Statistics Canada, 2011). Although this study will investigate aggregate-level SES, these statistics are informative in providing a picture of the population in Ontario and Canada.

2.3.1.2 Education

Education is a core marker of SES and has been increasingly considered the most important marker of SES. Education has been consistently suggested as one of the more important parameters of SES in epidemiological studies (Winkleby, et al., 1992). Education indicates the acquisition of skill sets required for obtaining economic resources, as well as positive social and psychological resources (Winkleby, et al., 1992).

Higher education may also be linked to better health outcomes by an increased knowledge of risk factors and the health care system, as well as the ability to apply the knowledge (Dall, 2006). Research suggests that there may be distinct health statuses at each education level. Education levels are categories of educational attainment such as: those who did not complete high school, completed high school but no post-secondary degree, and completed a college degree (Muller, 2002). Having a lower education encompasses resource deprivation, as well as having an occupation with increased risks of occupational injury and exposure and learned risk behaviours (Muller, 2002). Winkleby et al. (1992) studied SES and its contribution to risk factors for cardiovascular disease, and found that education was the only measure that was significantly associated with the risk factors ($p < 0.05$), and that if a single parameter needed to be chosen, higher education may be the best predictor of good health (Winkleby, et al., 1992). Higher risk was associated with lower levels of education in the Winkleby et al. study. Since their study investigated cardiovascular disease, the results are not necessarily generalizable to other diseases, however it provides insight into the importance of education on health outcomes.

As summarized in the Socio-economic Atlas 2006, there is a higher percentage of the population that have not completed a high school education in the Hamilton-Niagara-Haldimand-Brant region compared to the province of Ontario. Additional demographic information about education in the Niagara region include that 14% of people aged 25-64 years have not completed high school, compared to 13.6% in Ontario (Niagara Region, 2011).

2.3.1.3 Occupation

Occupation is also a core marker of SES. Occupation can be classified into many categories including managerial, administrative, professional, trade and technical occupations. Occupation has been considered to measure prestige, responsibility, physical activity and work exposures (Winkleby, et al., 1992).

2.3.1.4 Family composition

Family composition describes two-parent versus lone-parent households and may be a risk factor in children and adolescent's bone health outcomes. The contrast in income between different family compositions is stark: the median family income of Canadian two-parent families with both parents working was $79,000 and for single-parent, female-headed households was $30,400 (Willms, 2010). The percentage of lone female parents in the Niagara region is 21.9% compared to 20% in Ontario. The percentage of lone male parents in the Niagara region is 5.4% compared to 4.5% in Ontario (Niagara Region, 2011).

2.3.1.5 Housing

Housing tenure describes those who own or rent their dwelling or occupy band housing (example: shelter occupancy on reserves), and the aggregate variable for housing tenure describes the proportion in each census area occupying private dwellings by either owning or renting that dwelling (Statistics Canada, 2011).

2.3.1.6 Labour force

Unemployment rates are used as an indicator of SES. The unemployment rate defined by Statistics Canada is the total number of unemployed individuals 15 and older divided by the total number individuals 15 and older participating in the labour force (Statistics Canada, 2011).

2.3.1.7 Proportion/prevalence below the low income cut-off (LICO)

The proportion below the low-income cut-off is defined as the percentage of economic families or persons not in economic families who spend 20% more of their after-tax income than the average family on food, shelter and clothing. From a study by the Government of Ontario examining low-income families in the Niagara Region and cognitive development in the early years, it was found that 12% of children in the Niagara Region were living in low-income families (annual family incomes below $30,000). Their study suggested that although family income is not the only determinant of children's developmental outcomes, children living in poor economic circumstances face significant developmental challenges not experienced by other children (Willms, 2010). Although the aforementioned study focused on low-income families and cognitive development in the Niagara region, it nonetheless suggests the adverse effects associated with living in a low income family. According to the City of Hamilton, the prevalence of families below the low-income cut-off (before taxes) is 14.3% compared to 11.7% of families in Ontario (City of Hamilton, 2011). According to Statistics Canada, 9.4% of Canadians are in a family whose income is below the low-income after-tax income cut-off as of 2008. The low-income cut-off according to the Family Expenditures

Survey in 1992 was $21,359 for four-person families in medium-sized cities (Statistics Canada, 2011).

2.4 Population Density

Population density is defined by Statistics Canada as the number of people per square kilometre. Population density can impact living conditions such that thinly populated areas (or rural areas) may have less accessible resources and access to primary health care, and a higher at-risk-of-poverty rate, while more densely populated areas may have a higher crime rate and severe material deprivation (Eurostat, 2012). Severe material deprivation is influenced by the local cost of living and is usually higher in larger cities (Eurostat, 2012). An *urban area* is defined as an area with a minimum population concentration of at least 1,000 persons and a population density of at least 400 people per square kilometre. A *rural area* is classified as any territory outside urban areas (Association of Public Health Epidemiologists in Ontario, 2012).

2.4.1 Niagara Region

The Niagara region has a population density of 230.5 people/km² compared to 13.4 people/ km² in all of Ontario. Overall the Niagara region has a relatively large rural area. There are only four municipalities out of the twelve in Niagara that make up the region which have a higher population density than Niagara as a whole. These are Grimsby (which has a population density of 347.2/ km²), Niagara Falls (392.1/ km²), St. Catharines (1,373.3/ km²) and Welland (620.7/ km²) (Niagara Region, 2011).

2.4.2 City of Hamilton

The City of Hamilton has a population density of 438.9 people/km² compared to 13.4 people/ km² in all of Ontario (Hamilton Direct Info, 2011). This is a relatively large in contrast to the province of Ontario as a whole.

2.5 Socioeconomic status and mechanisms to health outcomes

SES can be connected to health via biological and physiological etiologic mechanisms and pathways. Socioeconomic disadvantage can lead to biological mechanisms, such as a disparity in dietary intake, that connect low SES to poor health (Chen, 2010). Understanding mechanisms helps identify sites for interventions (i.e., health promotion and prevention strategies). Risk factors that emerge early in life may have implications for disease later in life, and SES may be a potential precursor to disease, and affect physiological aging, including osteoporosis (Chen, 2010). SES influences health-related behaviours through health knowledge and attitudes (Fukuda, Nakamura, Takano, Nakao, & Imai, 2007). SES may alter the presence of modifiable risk factors for osteoporosis and low PBM (i.e. nutrition, exercise, smoking, alcohol consumption, etc) and therefore have an impact on the bone SOS among adolescent females.

Examples of how SES may alter the presence of risk factors for low PBM and osteoporosis are described below, for physical activity. In 2010, a study by Singh and Evans indicated that children from neighbourhoods with low SES exercised for shorter duration (Singh & Evans, 2010). Exercise for shorter duration results in lower levels of physical activity which can result in inadequate PBM attainment of children and adolescents.

In another study, Federico et al (2009) studied SES inequalities and physical activity practices among Italian children and adolescents aged 6 to17 years. They found that the children and adolescents whose parents held a middle or high educational title were 80% more likely to practice moderate or vigorous physical activity than subjects whose parents had a lower level of education (OR = 1.80, 95% confidence interval (CI) = 1.40 – 2.33) (Federico, Falese, & Capelli, 2009). These results indicate that parental SES plays a significant role in physical activity practice, and these results could also indicate a mechanism between SES and bone SOS.

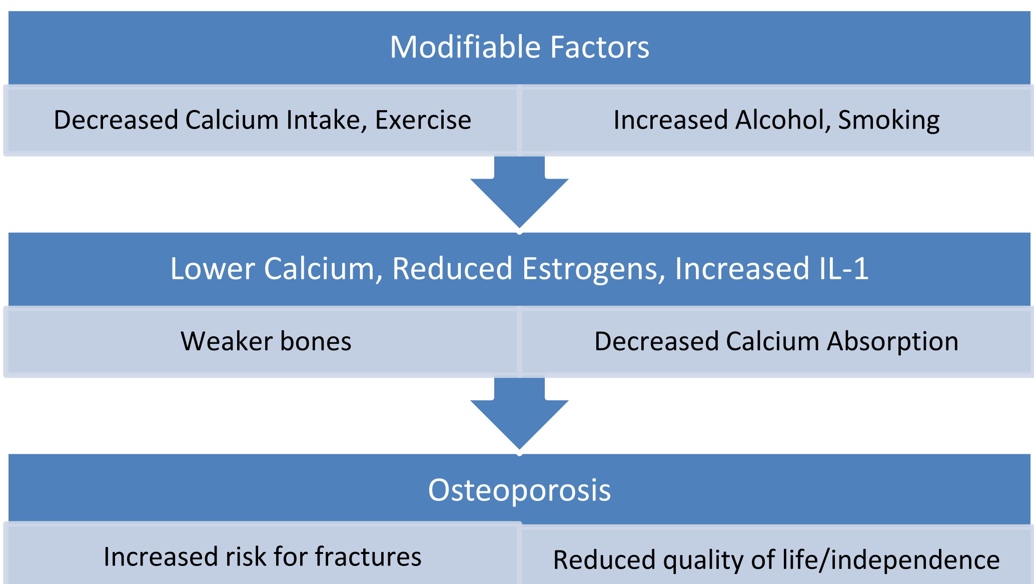


Figure 3: Modifiable risk factors affecting bone health: Mechanism to osteoporosis

2.6 School attended and mechanisms to health outcomes

The value of school in a child and adolescent's life has been established as critical. However, the extent to which school plays a role in a student's health, particularly bone health, requires further investigation. The impact and possible buffering effect school may have on an adolescent's health despite low SES background could provide insight into new prevention strategies targeted towards adolescents and their bone health. According to Murphy et al (2006), schools are considered to provide useful settings for promoting physical activity among health behaviours in adolescent females. This is attributed to the considerable time periods spent there, and the opportunity for formal health teaching by skilled teachers, staff encouragement and communication, and access to facilities and equipment (Murphy, et al., 2006). Although the relationship between the school environment and bone health has not been examined extensively, there has been some evidence suggesting that school impacts various types of health behaviours. An Australian study by McLellan et al (1999) examined the association between school environment and seven health behaviours, including tobacco use, alcohol use, nutritional intake and physical activity, among 3918 students in Years 6, 10 and 8 (grades) from 116 randomly sampled schools. Adjusting for age, sex and average weekly pocket money, students who had positive perceptions of their school environment and perceived their teachers as supportive were significantly more likely to participate in health promoting behaviours (McLellan, Rissel, Donnelly, & Bauman, 1999). This study provided evidence towards school impacting health behaviours, however, this study was conducted in Australia. A longitudinal study in Scotland by West et al (2004) also investigated school effects on student's health behaviours, using multi-level modeling, of 2196 students between 11 and 15 years of age. Higher levels of smoking and drinking were found in larger schools independently-rated as having a poorer ethos (or spirit of the school) and in schools containing more students with lower levels of involvement with their school and their teachers (West, 2004).

A two year school-based intervention by Gortmaker et al (1999) attempted to reduce obesity of 1295 ethnically diverse students in 10 public schools from grades 6 to 8 in Massachusetts. The intervention, using classroom sessions focusing on positive health behaviours such as physical activity and decreasing consumption of high-fat foods, were found to contribute to a greater remission of obesity among intervention girls versus control girls (OR = 2.16, 95% CI = 1.07 – 4.35) (Gortmaker et al., 1999). Interventions in schools targeting the reduction of obesity could also facilitate improving bone health outcomes by lowering high-fat intake and increasing physical activity, which also affect bone quality.

With this growing body of evidence of the impact of school on health behaviours, there may be a link between school attended and health behaviours affecting bone health. It is therefore important to study schools and their potentially critical effect on bone health in Canada. Six randomly sampled high schools from Southern Ontario were included in this study and are part of the analysis.

2.7 Gaps in the literature

Generally low SES is associated with poorer health outcomes (Brennan, et al., 2010) however there is limited literature on the relationship between SES as a risk factor and bone health among adolescent females, particularly among Canadian adolescent populations. Although previous studies have investigated and found links between SES and health outcomes, there is a particular lack of research on the association between SES, school and bone strength.

A review of the literature revealed that there is limited use of bone speed of sound (SOS) measurements by quantitative ultrasound (QUS) compared to the more popular use of bone mineral density (BMD) measurements for bone quality measured by dual-energy X-ray absorptiometry (DXA). For instance, Brennan and colleagues (2009) assessed BMD and its association with SES in a population-based sample of 1494 adult women in Australia. Brennan and colleagues found that there were patterns of low BMD in both ends of the SES continuum such that individuals in the upper SES quintile and lowest SES quintile had the lowest BMD (Brennan S. L, 2009). The pattern that individuals with low SES have poor bone health was supported, however, in this study BMD measured by DXA was the outcome studied (Brennan S. L, 2009), as opposed to using the outcome of bone SOS measured by QUS. Various other studies (Brennan, et al., 2010) examining the effect of SES have also focused on the outcome of BMD and predominantly in older adult populations. Bone speed of sound is an effective assessment technique of choice due to its ability to provide multiple characteristics of the bone such as measurement of speed of sound along the length of a long bone, and providing information not only about bone density, but also the micro-architecture, cortical thickness and elasticity of the bone (Omar, 2006). Since bone strength is determined from both its material and its structural properties, this additional information provides a summary of the numerous, interrelated components that impact bone strength (Davison et al., 2006). As stated by Davison (2006), in reference to bone strength, the whole is greater than the sum of its parts. Therefore further research investigating bone SOS as the outcome would be beneficial in order to enrich the literature pertaining to bone health.

In 2005, Wang and colleagues investigated the socioeconomic influences on bone health in postmenopausal women using cross-sectional data from NHANES III, 1988-1994. The results from this study indicated that education likely plays an important role in improving calcium intake among low-income women, and as well education and income were positively associated with BMD among Black and White postmenopausal women (M. C. Wang & Dixon, 2006). Although results from this study highlighted important socioeconomic differences in BMD among ethnic and socioeconomic groups of postmenopausal women, these results are only generalizable to older women.

Further research among adolescent females is required in order to investigate prevention strategies as opposed to treatments. Most of the studies investigating the association between SES and bone health focus on adult populations and on BMD, and less so on younger populations or bone SOS which captures greater information about bone quality. Of the few studies that examined children, including a study by Arabi and colleagues (2004), there appear to be critical study limitations. Arabi and colleagues examined bone mineral density and SES in healthy Lebanese children and adolescents, and found that children of high SES tended to have higher BMD values than those of lower SES of the same gender (Arabi et al., 2004). However this study had such low numbers in each subgroup that statistics were not even reported (the sample sizes for the low SES schools versus the high SES schools were not available). Additionally, this study was conducted in Lebanon, a nation vastly different from Canada therefore these results are not generalizable to Canadian populations.

Reinforcing these limitations in the literature, Akarirmak and colleagues conducted a study in Istanbul, Turkey, and compared bone SOS in 60 primary school children in two different schools of high and low socioeconomic status. Children from the higher socioeconomic level school were found to have higher SOS values however the difference was not statistically significant (Akarirmak, 1996). In addition to the small sample size of 60 children reducing the power of this study, schools in Turkey may also differ from schools in Canada, and therefore pursuing research in a North American region is important in order to contrast and possibly validate the research in European and Asian nations. Canadian adolescents including adolescents from the Niagara Region and Hamilton have not been studied extensively, and although the SES in these regions may not range in extreme values compared to nations overseas, about 1 in 6 Canadian children live in low-income families, and there are still many children in the Niagara Region and Hamilton living in low-income families (Willms, 2010).

Another gap in the literature is that the effects of school on bone SOS have not been studied extensively, particularly in Canada. School effects often represent school characteristics, such as school curriculum, meal and physical activity programs, which may affect child and adolescent outcomes. Although there is one Canadian study by Janssen and colleagues in 2006 examining the influence of area-level measures of SES on obesity, unhealthy eating, and physical inactivity among 6684 Canadian adolescents which utilized area-level measures of SES similar to our study, this study however focussed on obesity as the outcome, ignoring bone health as an outcome. All of the above gaps in the literature will be addressed in this study by examining the association between Statistics Canada 2006 aggregate-area based census-derived estimators of SES and bone SOS in a moderately large sample of Canadian female adolescents (n = 412), as well as differences in bone SOS among multiple schools.

CHAPTER 3: METHODOLOGY

This study was a secondary data analysis and utilized data collected from the Brock Osteo Nutrition and Exercise Study (BONES) conducted by researchers at Brock University. The study design, study sample, data collection and proposed data analysis procedures are described in this chapter. SES was the exposure variable under investigation. Outcome variables that were evaluated were primarily bone SOS. Additional variables were assessed for their possible placement between SES and bone SOS: calcium intake, physical activity, nutrient supplementation, alcohol consumption, and smoking. Variable measurements are described in the following subsections.

3.1 BONES protocol and study design

BONES was a cross-sectional study that recruited adolescent girls from six randomly sampled high schools from the Niagara Catholic School Board and the Hamilton-Wentworth School Board between 2007 and 2008. This study was originally approved by the Research Ethics Board at Brock University and by two school boards, Niagara Catholic District School Board and Hamilton-Wentworth District School Board. Participants and their parents/guardians provided written informed consent prior to participation in the BONES study. For BONES, socio-demographic and bone QUS data were collected for 412 adolescent females in grades 9 - 12 from six randomly selected high schools out of 26 possible secondary schools in the participating two school boards during two assessment visits. During the first visit, participants completed the questionnaire package. The BONES questionnaire was administered to all participants (refer to **Appendix 1**). Address data were extracted from these questionnaires and participant address data were matched to aggregate SES variables from Statistics Canada 2006 census-derived SES data. From data provided by Statistics Canada 2006 census data, selected SES aggregate indices were chosen as possible candidates for study as exposure variables. These were median household and family income, employment rate, and proportion below the low-income cut-off.

Anthropometric measurements were administered and bone SOS of the non-dominant and dominant tibia and radius were assessed using a portable QUS device brought to the schools as a measure of bone health during the second visit.

3.2 Participant recruitment

Participants were recruited from their regularly scheduled physical education classes from the identified randomly chosen schools. An information visit with the principal and physical education teachers was set up to discuss the study requirements. An information letter and informed consent forms were then distributed to students and their parents, and a signed informed consent was obtained from all participants and their parents. Participation rates for the classes were examined to identify potential selection bias.

3.3 Study sample

In order to match participant with aggregate level SES, a complete address for each participant was required. Of the original 441 participants with address data available, data for the participants recruited solely from a rowing (n = 19) or swim team (n = 10) and not from a school were excluded from analysis, due to the school focus of the analysis, thereby excluding twenty-nine female adolescents. Exclusion of these participants preserved the sampling of the study. The study sample size consisted of 412 adolescent females in grades 9-12 with complete address data. Of those with address data, 368 adolescent females had non-dominant tibial SOS data, therefore the final analytic study sample size was 368 adolescent females.

3.4 Measurements

3.4.1 Bone speed of sound

Bone speed of sound (SOS) was the outcome variable of interest and was measured by transaxial quantitative ultrasound (QUS). Speed of sound travels faster through non-porous solids and liquids than through air, therefore greater bone mass will result in a faster speed of sound value. In bone, the speed of sound waves that propagate along the cortical bone are measured in metres/second (Foldes, et al., 1996). Speed of sound results are usually expressed as Z-scores which are units of standard deviations from age and sex matched normal mean values (Foldes, et al., 1996). However, for this study speed of sound results were expressed in their raw units of meters/second in order to provide specific effect estimates. For BONES, QUS measurements on four anatomical sites, the mid-tibia and distal radius, for both dominant and non-dominant sides, were conducted by two trained investigators with no significant differences in the average SOS scores measured by each investigator. The intra-class correlation coefficient (ICC) between the two investigators' measurements for the four skeletal sites ranged from 0.81 to 0.88.

Quantitative ultrasound (QUS) is a technique gaining greater attention for assessing bone status, partly for its ability to measure bone SOS at multiple skeletal sites (Weiss, Ben-Shlomo, Hagag, & Rapoport, 2000) and for its ability to provide information about a multitude of bone properties such as bone mineral density, micro-architecture of the bone, cortical thickness and elasticity. Advantages of this technique are that it can be performed quickly, is relatively inexpensive, is portable, and involves no radiation due to being ionizing-radiation free (Njeh et al., 2001). QUS provides a bigger picture of the numerous, interrelated components that impact bone strength (Davison, et al., 2006). This is in contrast to the more commonly used method of assessing bone health by the dual-energy X-ray absorptiometry (DXA) device. DXA measurements assess bone mineral density, only one component contributing to bone health. DXA can provide accurate reproducible measurements of BMD from experienced technicians, however this device is operator-dependent, and there are multiple opportunities for error (Richmond, 2003) . As well, there is exposure to radiation by use of X-ray technology. The QUS device utilized for this study was the Sunlight Omnisense (Sunlight Omnisense 7000S, Sunlight Medical, Israel).

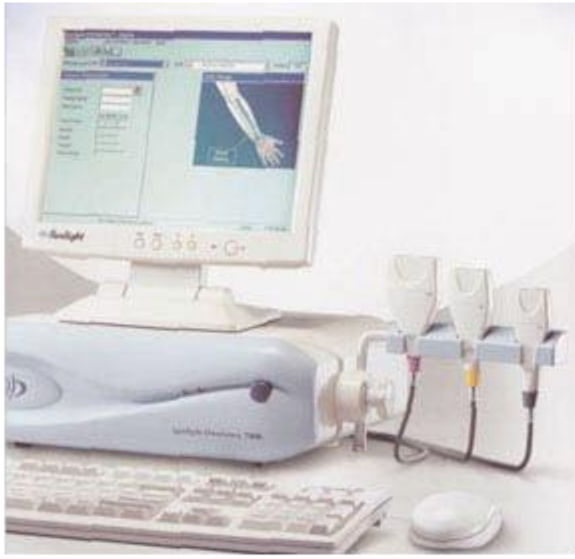


Figure 4: QUS Sunlight Omnisense device

3.4.2 Outcome variables: Non-dominant tibia

Bone SOS scores for the dominant and non-dominant tibia (r = 0.77) were moderately correlated, and correlation was slightly higher for the tibia than correlation of bone SOS scores for the dominant and non-dominant radius (r = 0.67). The non-dominant side for the mid-tibia and distal radius generally experiences less usage and therefore there is less variation in the non-dominant bone compared to the dominant side, where usage is greater and more variable. The mid-tibia shaft is part of the bone found below the knee. Advantages of assessing bone SOS using the tibia bone include its ability to show more weight-bearing adaptations of bone from physical activity, compared to the radius, which is found at the lateral aspect of the wrist. As well, measurement of tibial SOS was suggested by Foldes (1995), who assessed the use of QUS of the tibia on 307 adult women in Israel, to be a precise method of assessing bone status (without exposing the participant to radiation) (Foldes, Rimon, Keinan, &

Popovtzer, 1995). Therefore the non-dominant tibial SOS scores were utilized as the outcome variable in the analysis.

3.4.3 Anthropometric measurements

All anthropometric measures were made by the same trained researchers for all participants. Height (without shoes) was measured to the nearest 0.1 cm. Body mass (kg), lean mass and relative body fat (% body fat) were measured using the InBody520 Bioelectrical Impedance Analysis (BIA) machine (Biospace Inc., Beverly Hills, USA).

3.4.4 BONES questionnaire package

The BONES questionnaire package consisted of standardized questionnaires that assessed leisure time physical activity and daily calcium intake. General information was collected through this package such as demographics, medical information, menstrual history (age of menarche and regularity of cycle), and use of oral contraceptives. The Godin-Shephard Leisure Time Exercise Questionnaire (G. Godin & Shephard, 1985), a self-report method, was part of the package and assessed physical activity by the weekly physical activity metabolic equivalent (WPAeq), and has demonstrated adequate validity and reliability in adolescents (Wolf et al., 1993). The questionnaire asked participants how many times they participated in mild, moderate, and strenuous physical activity for at least 15 minutes in their free time in the past 7 days. These numbers were summed after first multiplying them by metabolic equivalent values ([METs] – 9 for strenuous, 5 for moderate, 3 for mild exercise), resulting in the total weekly activity (WA_{eq}). Active (substantial benefits) was considered 24 units or more, moderately active was between 14 and 23 units, and less than 14 units was considered insufficiently active (Gaston Godin, 2011).

The rapid assessment method (RAM) (Hertzler, 1994), a self-report method that has been previously used to assess calcium intake in young women, was used to assess daily calcium intake. This questionnaire asked participants to record the number of servings of calcium in one typical day in the last seven days. The total milligrams of calcium consumed in one day was subsequently assessed and calculated. All questionnaires were administered by the same researchers.

3.5 Data analysis using AABCDEses

To undertake the current analysis, an application to the research ethics board for the secondary use of data was submitted and approved. See **Appendix 2**.

3.5.1 Exposure variables: Socioeconomic status

Aggregate area-based census-derived estimators of SES were evaluated as the distal exposure variables in this study as opposed to individual–level measures of SES. In this current study, due to a less than 30% return of parental questionnaires (129 parental questionnaires out of 441 parental questionnaires were returned, as reported by investigators), and adolescents being uninformed of their parent’s income (391 participants reported “do not know” for yearly household income), accurate and complete individual-level SES data were unavailable. At the individual level, education is considered a good indicator of health status, as mentioned in Chapter 2. However, there was also substantial missing data and underreporting of parental education and occupation. The majority of adolescents did not report or did not have an accurate answer to this question on the questionnaire. However, addresses of 412 participants (441completed student questionnaires with addresses minus 29 students recruited solely from row and swim teams) and their parents were available, and therefore the use of aggregate SES measures was particularly useful for this investigation.

3.5.2 Residential addresses

If the participant's parents had separate addresses, the mother's address was utilized due to *a priori* reasoning that the mother is most often the primary guardian after parental separation.

3.5.3 Geo-coding and matching addresses to census areas

In this investigation, since SES aggregate variables were used, it was necessary that addresses were geo-coded from the full addresses provided. The census area unit used in this study was a Dissemination Area (DA), the smallest standard geographic area unit with populations of approximately 400-700 persons (Statistics Canada, 2011). Each participant's residential address and school addresses were geo-coded and placed in its corresponding dissemination area (DA). Geo-codes were obtained for the 2006 Census dissemination area boundaries. In order to geo-code, latitude and longitude coordinates were obtained for each address. Latitudes and longitudes were placed within dissemination area boundaries for the 2006 census year. Each participant address was assigned an appropriate tract number, and then linked with DA level SES indicators. In summary, SES was determined by matching participant residential address to aggregate

SES variables from Statistics Canada 2006 Census data for her dissemination area. Geo-coding and matching addresses to census areas were carried out by a professional trained medical geographer, Mr. Ryan Waterhouse, at the Niagara Region Department of Public Health.

3.5.4 Selected AABCDEses variables

From data provided by Statistics Canada 2006 census data, selected AABCDEses variables were studied as potential distal exposure variables. These were initially median household and family income. Income is a simple but powerful and effective predictor of health, particularly at the aggregate level, as there is usually underreporting or inaccurate reporting of income at the individual level. Median household income is one of the main AABCDEses for income by Statistics Canada. A household is defined as a person or group of persons residing in a dwelling. Median family income makes use of the economic family, which as defined by Statistics Canada includes two or more persons living in the same dwelling who are related to each other by blood, marriage, common-law or adoption, including foster children. Family income is the sum of income of each adult in the family as defined above. Household income is the sum of incomes of all adults in the household (Statistics Canada, 2011). The median family or household income is the dollar amount at the midpoint of a distribution of families or households ranked by the size of family or household income (Statistics Canada, 2011). Median family income and median household income were found to be highly correlated and reasoning for the decision to choose one of these measures is described in Chapter 4.

3.5.5 School SES

School SES was determined for each school as the AABCDEses for the DA that the school address was located.

3.6 Statistical Analysis and Analytic Strategy

Statistical analyses were performed using STATA 11 and STATA 12 (College Station, Texas, USA). Data were cleaned, assessing for missing values and removing participants whom were recruited solely from row or swim teams in order to eliminate selection bias, which might occur from this inclusion. An overview of the analytic approach is described below.

3.6.1 Study objective/Research question - Examine the association between SES, schools and bone health in adolescent girls as measured by SOS.

In order to investigate the association model and thus potential causal mechanism, the relationship between distal factors, proximal factors and bone SOS was examined. Distal variables were AABCD estimators of SES. Proximal variables were individual intermediate risk factors including calcium intake, regular nutritional supplementation, physical activity, % body fat, regular smoking and regular alcohol consumption. Univariate analyses were conducted to examine the association between distal and proximal variables and bone SOS. Furthermore, the associations between AABCDEses variables and proximal variables to bone SOS (i.e. calcium intake) were examined. Finally, multivariable analyses between AABCDEses variables and bone SOS were examined.

Descriptive statistics were evaluated including measures of central tendency such as means, and measures of dispersion such as standard deviations and ranges. Multivariable regression models were employed to assess the association between SES indices and bone SOS at the anatomical site of the mid-tibia, for the non-dominant side. Unadjusted and adjusted models evaluated which models best explain variation in bone SOS. Certain variables needed to be included in the models as potential confounders. A confounder is a third factor that is associated with the risk factor under investigation, as well is associated with the outcome of the study, however does not lie in the causal pathway between the risk factor of interest and the outcome of interest, in this case between SES and bone SOS. A possible confounder included age. Multivariable regression analyses were used to assess the association between SES indices and bone SOS in a multilevel model with participants nested in schools. Refer to **Figure 5** for hypothesized causal mechanism between SES and bone SOS.

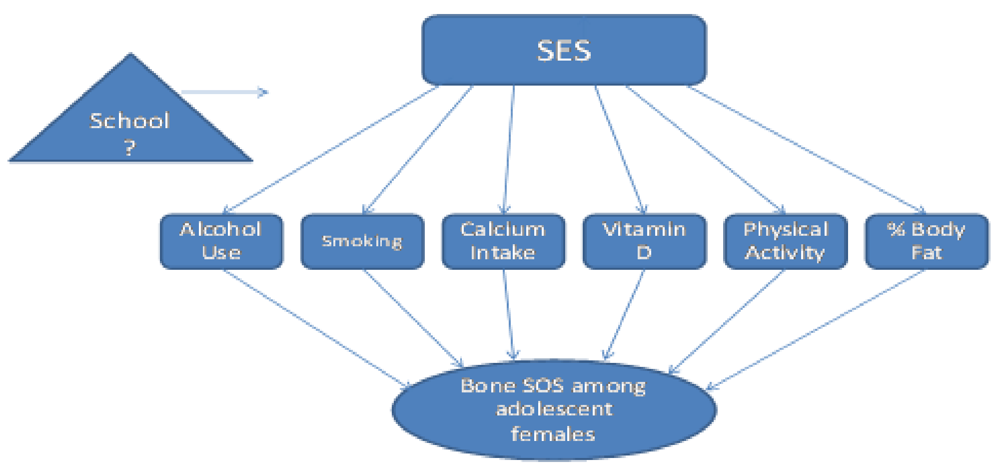


Figure 5: Schematic representation of possible relations between distal factors SES and school effects, proximal factors to bone SOS (i.e., alcohol use, smoking, calcium intake, physical activity, percent body fat) and bone SOS in adolescent females

3.6.2. Multilevel modeling

Multilevel modeling simultaneously examines the effects of group-level and individual-level factors on individual health outcomes, allowing for a contextual analysis, and a more accurate identification of at-risk populations (Chaix & Chauvin, 2002; Martinez et al., 2009). It helps explain variance when there is a hierarchical structure i.e. individuals within groups (Hox, 1998). Not adjusting for clustered data may lead to the under-estimation of standard errors, confidence intervals, and p-values (Hox, 1998). There are two main reasons for applying the multilevel model concept, firstly, for statistical reasons to improve the estimations of the standard errors, and secondly, epidemiological reasons, for exploring variance, i.e., how much of the individual differences are between schools? (Pittsburgh, 2010).

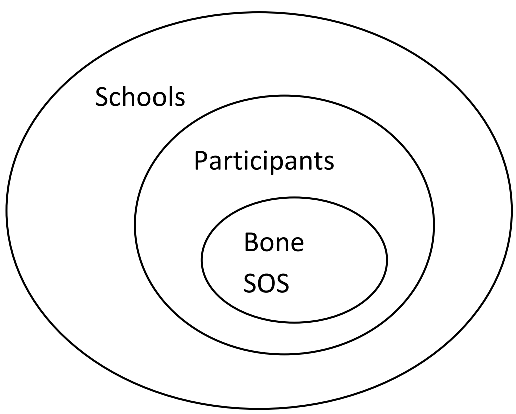


Figure 6: Schematic diagram of multilevel model: Participants/students level nested in school level; investigating variance in students' bone SOS

There are two types of multilevel models, the random effects model and the fixed effects model. The random effects model examines the between-group variation (intercept) and within-group variation (residual) by treating the group-level as the random effect, in this study, where the participant's school is the random effect. In the fixed effects regression models, the group effects are treated as fixed effects, so that estimation is straightforward, with the indicator variable for the higher level, or cluster, included as a categorical factor in the regression analysis (Fitzmaurice, 2005). In this study, the intercept value was for the baseline school.

For this study, multilevel multivariable regression analysis examined students nested within schools, assessing bone SOS as the outcome variable. In most random effects models, a large number of groups, or clusters, are required. Therefore the fixed effects model was utilized as this study involved six schools. In this study, participants are naturally clustered in schools, such that school effects could be revealed, where school effects represent unobserved school characteristics that affect adolescent outcomes. Correlations between outcomes for students from the same school are attributed to these unobserved variables.

Accounting for clustering was also conducted due to a possible clustering of similar participants within schools, where there might be intra-cluster correlation and less variation between participants within a school, violating the assumption of independent observations (Wears, 2002). The observations of participants within the same school could be correlated, due to common school-specific characteristics such as physical activity programs and other programs promoting positive health behaviours. Failure to account for the natural clustering and the dependence between individual observations could result in p-values and standard errors that are too small (Wears, 2002). As intra-cluster correlation increases, Type I error rates can increase considerably (Dorman, 2008). In regards to our study, there is a clustering of participants in schools, and these participants are likelier to have similar characteristics and less variation in bone SOS. Therefore, accounting for clustering was conducted by including the **cluster (School)** command in the regression syntax in STATA analysis. This was used in order to correct and obtain robust standard errors and accurate p-values, when fixed school effects were not included in models.

3.6.3 Multiple imputation analysis

In order to handle missing values, the multiple imputation method was utilized to replace missing values with multiple sets of simulated values in order to complete the data set. Data imputation was used to estimate the values of missing covariates (Dupont, 2009), and using multiple imputations to estimate the values of missing covariates and confounding variables helped minimize bias in a sample (Dupont, 2009). The multiple imputation method simulated the dispersion of the missing values thereby obtaining more accurate estimates of the standard errors of the model coefficients (Dupont, 2009). In contrast, the limitation behind the single imputation method is that the imputed values are treated as though they were observed when actually imputations are only estimates. Thus, the single imputation method can lead to biased underestimation of standard errors (UCLA Statistical Consulting Group, 2011). Theoretically, the idea behind multiple imputations is to replace each missing value with a set of plausible values therefore creating multiple completed data sets (Rubin & Schenker, 1991). The completed data set is then analyzed using standard complete-data methods. Building an imputation model requires several decisions, including what method should be used to generate imputations; whether to impute for a specific analysis model, or to impute for an entire dataset; whether any auxiliary variables should be included in the imputation model; and lastly, how many imputed data sets should be generated (UCLA Statistical Consulting Group, 2011).

After assessing the BONES data set, and observing that calcium intake had 201 missing values (49%), the multiple imputation method was implemented, and missing values for the variable calcium intake were imputed. Missing data for calcium intake occurred due to incorrect and inaccurate responses by participants which could not be included in the data set. Many participants misunderstood whether they should answer their calcium intake as ‘per day’ or ‘per week’. These inconsistencies resulted in excluding the data for calcium intake for those participants from the dataset, resulting in missing data for those participants. Using STATA 12, the **mi** command was utilized to simulate values for the missing values of the variable calcium intake. Other variables were imputed however were not included in final models.

For this study, a series of ten imputed datasets were generated. A larger number of imputations are often recommended to ensure accuracy of estimates, and may also allow hypothesis tests with less restrictive assumptions. Usually between 5 and 20 imputations are recommended for low proportions of missing data, and when the proportion of missing data is higher, up to 50 or more imputations are recommended (UCLA Statistical Consulting Group, 2011). The imputed data sets were modeled separately and results were pooled according to the method of Rubin (Rubin, 1996) using the STATA command **mi estimate.**

Since the coefficients of determination (R^2) were not provided when running regression models using the **mi estimate** command with multiple-imputed data, the coefficients of determination were obtained by generating an R^2 for each of the ten imputed data set. Study characteristics and descriptive statistics (Table 1 – Table 3) were computed from the original dataset, and not the multiply-imputed dataset. Results that employed the multiply-imputed datasets were any univariate analyses, multivariable regression analyses, or likelihood ratio tests that included calcium intake in the analysis.

3.6.4 Multivariable linear regression

Association models evaluated the associations between variables using multivariable linear regression with SOS as the outcome. Model selection was first based on *a priori* reasoning considering variables possibly related to the outcome, and then carried out using backwards elimination, using a p-value > 0.2 to eliminate variables from the model. Non-linear effects were analyzed and the assumptions of linear regression were tested.

3.6.5 Testing the assumptions of linear regression

The five principal assumptions of linear regression were tested in order to ensure that estimates from analysis are unbiased and efficient. If there were serious violations of any of these assumptions then corrective measures were taken as discussed below.

1. Linearity

The first assumption is that there is a linear or straight-line relationship between the independent and dependent variable. Possible combinations of continuous variables were visually examined by use of bivariate scatter plots to ensure this assumption was met. If this assumption was violated and a non-linear relationship was observed, then the independent variable was transformed in order to approximate a linear relationship, or restricted cubic splines were used to describe the non-linear association.

Locally weighted scatter plot smoothing (LOWESS)

The locally weighted scatter plot smoothing (LOWESS) is a procedure designed to smooth a scatter plot as an alternative to the classical procedure of polynomial smoothing. According to STATA 11, the procedure adds a locally weighted scatter plot smoothing of the plotted points to assist in detecting nonlinearities. The LOWESS procedure is considered more resistant to extreme data points than polynomial smoothing because the polynomial smoothing method of fitting curves with the least squares method can be affected by extreme data points on one side of the scatter plot, affecting the fitted values on the other side of the scatter plot. LOWESS regression focuses on the local points such that the smoothed value of y corresponding to data point x_i is obtained on the basis of the data points around it within a certain bandwidth. The band has the point x_i as its midpoint, and x_i has the highest weight, with the weights for the other data points within the band declining with their distance from x_i (according to a weight function). This method finds fitted values corresponding to x_i as the smoothed value, and is repeated for all the data points (Thinking with Data, 2011). In this study, the LOWESS procedure was used to assess non-linear trends, using the statistical software STATA 11.

Non-linear associations and use of restricted cubic splines

SES and continuous variables (i.e., median family income, BMI, % body fat) may exhibit non-linear relationships. Significant relationships may be observed using transformed variables whilst being insignificant in linear models. Quadratic transformations may prove non-linearity if significant, however since quadratic transformations do not easily change shape, restricted cubic transformations is the method of choice for correcting non-linearity. Restricted cubic splines facilitate evaluating non-linear relationships and can be used to correctly model the relationship between the exposure variable and the response (Harrell, Lee, & Pollock, 1988). Available information can then be extracted from a continuous exposure variable, thereby offering more accurate prediction, better control of confounding, and more powerful statistical tests (Harrell, et al., 1988). A spline is defined as a curve which connects two specific points (i.e. knots). Specifically, splines are smooth functions that can take on virtually any shape (Harrell, et al., 1988). They are called cubic splines because they are third order polynomial. This analysis generally consisted of the standard four knots and three splines using STATA 11. Four or five knots are usually adequate and altering knot placement usually does not greatly affect fit, therefore knots are usually placed automatically at fixed percentiles of the predictor as recommended by Harrell (Harrell, et al., 1988). Restricted cubic splines place an additional restriction that the function be linear in the tails (Harrell, et al., 1988). As well, a restricted cubic spline function in k knots requires estimating k-1 regression coefficients, unlike one coefficient if linearity is assumed (Harrell, et al., 1988). Restricted cubic splines are often chosen over ordinary cubic splines due to the instability in the tails of the fit of ordinary cubic splines before the first knot or after the last knot.

2. Mean independence

Mean independence refers to the independence of the errors. This assumption can be violated when potential predictor variables are omitted from the model, and therefore become part of the error term, and may cause the errors to be correlated with the dependent variable. This problem may be averted by including all important variables in the model.

3. Homoskedasticity

Homoskedascticity assumes that the variance is similar at all points along the regression line (Norman, 2008). This can be seen by construction of a scatter plot for a predictor and the dependent variable and evaluating whether the points seem to be evenly distributed above and below the regression line along its entire length. To examine this assumption further, the distribution of residuals were examined by plotting the residuals against the predicted values. The points should appear to be randomly distributed above and below the value of 0 with no apparent pattern (Norman, 2008). Deviations from a random scattering of points between ± 2 SDs presents heteroskedascticity, which can potentially bias the estimate of the standard error, and requires transformations of data. Homoskedasticity of models was tested using the **rvfplot** command in STATA.

4. Multicollinearity

Collinearity occurs when two independent variables are highly correlated in the range of r = 0.90 or higher (Norman, 2008). This can wash out the effect leading to the model showing variables as non-significant when both independent variables are in the model. This assumption was examined by use of bivariate analysis. If two independent variables were too highly correlated, only one of them was included in analysis.

5. Normality of the error distribution

This assumption assumes that the errors will be normally distributed. Excessive skewness, where the errors are not symmetrically distributed, and excessive kurtosis, where the errors deviate into an S-shaped pattern, was examined. With fewer cases for analysis this assumption becomes more important. The normality of the error distribution was examined using the **qnorm** command for predicting residuals in STATA however since this study has a moderately large sample size it was expected this assumption would be met. If not, non-linear transformation of variables was the solution of choice.

3.6.6 Interaction analysis

Interactions which may modify the effect of the SES exposure variables on bone SOS were analyzed. There have been very few studies analyzing the effect of the interaction of another exposure variable with SES on bone SOS, and in fact, only one study was found, which examined the effect of interaction of race and SES on bone SOS. Race data were not available in the current study. However possible interactions, such as the effect of an interaction of the variable family history of osteoporosis with SES on bone SOS, were examined and tested for statistical significance.

3.6.7 Multivariable logistic regression

Multivariable logistic regression was employed in order to predict the probability of low bone SOS versus high bone SOS, controlling for key covariates. The continuous outcome variable for non-dominant tibial SOS was dichotomized to create a dependent variable for logistic regression. The variable ndSOSt, describing the speed of sound for the non-dominant tibial bone, was divided into low bone SOS and high bone SOS, with those below the cut-point of the 25th percentile, at a value of 3763 m/s SOS, having potentially unhealthy bones. The reasoning for dichotomizing non-dominant tibial SOS at the 25th percentile was that since one in four women are likely to develop osteoporosis, it was likely that participants below the 25th percentile of non-dominant tibial SOS were considered to have poor bone SOS. Evaluating the results of multivariable logistic regression facilitated assessing the occurrence of low bone SOS.

In summary, statistical techniques used to analyze the data included multilevel modeling, univariate analyses, multivariable linear and multivariable logistic regression, interaction analysis, analysis of non-linear effects, as well as descriptive statistics in order to assess the associations being investigated in the study aims. All analyses accounted for the clustering effect of participants within schools. Likelihood ratio tests (LRT) assessed whether one model fits better than another (the null or the alternative model), and whether a specified variable contributed significantly to the model. Thus, the LRT was used to test the significance of contribution of one or more predictor variables in nested models. In situations where the LRT could not be applied (example, in models accounting for clustering), Wald p-values were utilized by using the **test** command in STATA in order to evaluate the contribution of sets of variables.

CHAPTER 4: RESULTS

Outline of results

The results of this study presented in this chapter are as follows: descriptive statistics, followed by univariate analyses, assessment of non-linear trends utilizing the LOWESS procedure, multivariable linear regression models with use of likelihood ratio tests and Wald test *p*-values, interaction analyses, and multivariable logistic regression modeling. The effect of clustering of participants in schools was accounted for in univariate and multivariable regression models. Post-estimation tests of assumptions of linear regression are also presented.

4.1 Sample characteristics

Basic characteristics and descriptive statistics including means, standard deviations and ranges for this cross-sectional study sample are presented in **Table 1** and **Table 2**. Address data and therefore SES data were available for 412 participants. Data for non-dominant tibial SOS were available for 368 participants. The mean age was 15.7 (SD 1.0) years, with a range of ages between 14 and 20 years. The mean BMI was 22.4 kg/m^2 (SD 4.1) and the mean percent body fat was 27.8% (SD 8.3). Mean calcium intake was 1306.6 mg (SD 469.4). The mean non-dominant tibial SOS was 3822.52 m/s (SD 100.60). Of study participants, 10.7% reported regular smoking, and 20.2% reported regular alcohol consumption. As presented in **Table 1,** the average of all the median family incomes of the dissemination areas of the sample was $68,162 (SD $19,366).

Table 1. Descriptive characteristics of selected variables for the study sample in BONES, 2007-2008

Variable	N	Mean (SD)	Range
Age (years)	410	15.7 (1.0)	14 – 20
Grade	412	10 (0.9)	9 – 12
Weight (kg)	408	59.2 (11.4)	33.6 – 103.2
Height (cm)	411	162.2 (6.4)	131.5 – 184.0
BMI (kg/m^2)	408	22.4 (4.1)	15.1 – 50.0
Percentage body fat	405	27.8 (8.3)	10.4 – 54.4
Calcium intake (mg)	211	1306.6 (469.4)	130.0 – 2260.0
Physical activity (Total Exercise Score)*	406	55.3 (29.6)	0 – 203
Bone SOS (m/s)			
Non-dominant tibial	368	3822.5 (100.6)	3497 – 4054
Regular smoking N (%)	410		
Yes	44 (10.7)		
Regular alcohol consumption N (%)	410		
Yes	83 (20.2)		
Family history of osteoporosis N (%)	395		
Yes	39 (9.9)		
Median family income N (%)	412	68,162 (19,366)	27,933 – 121,254
< $40,000	30 (7.3)		
$40,001 - $60,000	126 (30.6)		
$60,001 - $80,000	139 (33.7)		
➢ $80,000	117 (28.4)		

Abbreviations: BMI, body mass index; n, sample size; SD, standard deviation; SOS, speed of sound.
*Total Exercise Score was measured by Physical Activity Questionnaire in BONES Questionnaire Package, assessing weekly physical activity levels.

Table 2 describes the characteristics of the students attending each of the randomly selected schools for the BONES study. School ranking from highest mean student SES to lowest mean student SES as measured by median family income ranked first BT ($82,828; SD $13,714), followed by ND ($77,228; SD $ 19,450), SF ($75,486; SD $22,624), LS ($63,631; SD $14,858), SM ($58,549; SD $11,527) and WC ($56,328; SD $13,192). **Table 3** displays school ranking by non-dominant tibial bone SOS and ranked first was LS, followed by BT, ND, SM, SF and WC. School ranking by school SES ranked BT first, followed by SM, ND, LS, WC and SF.

Table 2. Characteristics of study students by school attended, BONES, 2007-2008.

School	n	Age (mean, years)	Grade (mode, R = range)	% Body Fat (mean)	Student SES (mean)*	School SES†	Bone SOS (mean, m/s)
BT	24	15.5 (SD 0.83; R 14.1 – 16.9)	10 (R 9-11)	24.9 (SD 8.1; R 11.2 - 39.6)	$82,828 (SD $13,714; R $56,101-$101,454)	$86,808	3852 (SD 91.5; R 3667-4054)
LS	67	16.4 (SD 0.77; R 15.2 – 18.1)	10 (R 10- 12)	30.5 (SD 8.9; R 15.4 - 51.4)	$63,631 (SD $14,858; R $32,631-$111,333)	$57,618	3856 (SD 92.7; R 3639-4011)
ND	116	15.7 (SD 0.99; R 14.0 – 19.0)	11 (R 9-12)	25.6 (SD 7.0; R 10.4 – 45)	$77,228 (SD $19,450; R $33,340-$115,652)	$73,193	3851 (SD 78.4; R 3649-4041)
SF	63	15.4 (SD 1.0; R 14.0 – 17.7)	9 (R 9-12)	26.3 (SD 7.4; R 12.1 – 54)	$75,486 (SD $22,624; R $27,933-$121,254)	$54,252	3818 (SD 96.2; R 3595-4012)
SM	44	15.1 (SD 0.95; R 14.1 – 16.9)	9 (R 9-11)	26.4 (SD 9.0; R 12.2 – 54.4)	$58,549 (SD $11,527; R $35,118-$86,786)	$75,985	3819 (SD 81.2; R 3612-3955)
WC	96	15.7 (SD 1.1; R 14.4 – 19.2)	9 (R 9-12)	30.8 (SD 8.2; R 11.4 – 48.4)	$56,328 (SD $13,192; R $29,791-$86,390)	$54,515	3747 (SD 116; R 3497-3967)

Abbreviations: DA, dissemination area; R, range; SD, standard deviation; SES, socioeconomic status; SOS, speed of sound.

*SES measure was median family income based on Dissemination Area.

†School SES was determined as the median family income within the DA which the school address was located.

Table 3. Schools ranked from highest (1) to lowest (6), for student SES, school SES, and non-dominant tibial SOS

Ranking	Student SES	School SES	Bone SOS
1	BT	BT	LS
2	ND	SM	BT
3	SF	ND	ND
4	LS	LS	SM
5	SM	WC	SF
6	WC	SF	WC

The school with the highest non-dominant tibial SOS was LS which was fourth in ranking for both student SES and school SES. This was unexpected and it was observed that LS also had the oldest study sample (mean age 16.4 years; SD 0.77). The school BT ranked second highest in non-dominant tibial SOS and had the highest student SES and also school SES. The third ranked school for non-dominant tibial SOS was ND and ranked second, and third for student and school SES, respectively. The lowest ranked schools for non-dominant tibial SOS ranked lowest for SES. These are univariate observations.

4.2 Pre-estimation testing for assumptions of regression modeling

Correlation analyses and testing the assumption of linearity were conducted. The assumption of linearity was conducted by checking for non-linear relationships between continuous exposure variables with the continuous outcome variable non-dominant tibial SOS. Testing these assumptions facilitated model building.

4.2.1 Correlation analyses

Possible collinearity between selected variables was assessed by Pearson correlation coefficients (table not shown). Age and grade were significantly positively correlated (r = 0.92, p = 0.0001, n = 410). Therefore for the most precise analysis and due to biological relevance, age was the covariate included in the adjusted regression models. Percent body fat was significantly positively correlated with BMI (r = 0.86, p = 0.0001, n = 405).

The correlation coefficient between median household income and median family income was also found to be significantly positive with a value of r = 0.87 (p = 0.0001, n = 412). Therefore for the purposes of this study, and due to using multiple SES indices creating problems due to multiple comparisons, only one SES index was utilized for this study. As stated by Winkleby et al., *"using multiple or composite measures may not significantly explain more about a population than would a single, well-chosen parameter"* (Winkleby, et al., 1992). Median family income and median household income were highly correlated, therefore median family income was chosen as the SES variable investigated in this study, based on the reasoning that adolescent females reside with their families. Therefore it was appropriate that median family income be utilized as the main well-chosen SES measure. Median family income as the only aggregate area-level SES index served as a simple and important predictor of bone health status and also minimized collinearity.

School SES and median family income exhibited a correlation coefficient value of 0.25 (p = 0.0001, n = 412) and therefore were not considered highly correlated or collinear. A variance inflation factor (VIF) value of 1.02 between School SES and student SES further indicated that School and SES were not collinear and therefore collinearity was avoided.

4.2.2 Non-linear analyses and use of restricted cubic splines

The following figures present LOWESS curves, assessing the non-linear relationship between continuous explanatory variables with bone SOS, such as median family income, BMI and % body fat. If a non-linear relationship was observed, then restricted cubic splines were created for those variables for use in analyses.

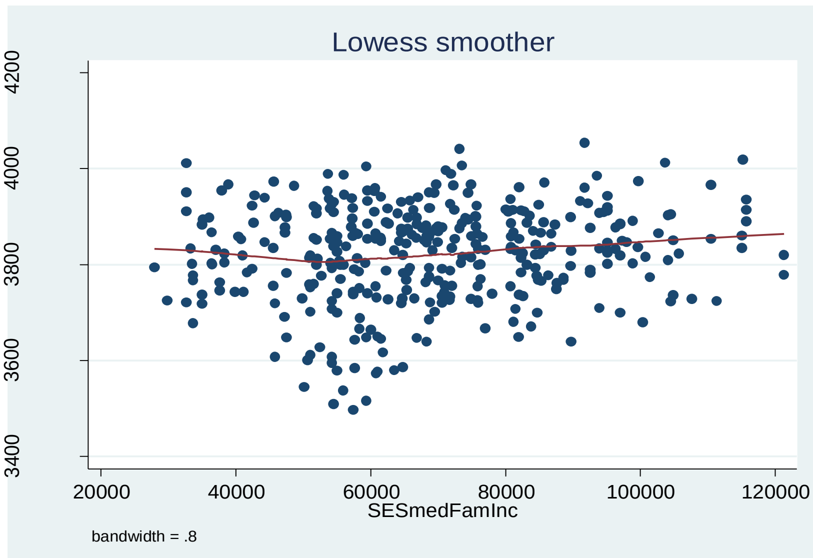


Figure 7: LOWESS curve for median family income and non-dominant tibial bone SOS

In **Figure 7**, a slight non-linear relationship was visible between the explanatory variable median family income and non-dominant tibial SOS. A negative relationship was exhibited up to approximately $55,000 and thereafter a positive relationship was exhibited between the two variables. Restricted cubic splines were created with four knots and three polynomial segments. Knots were located at the following locations: Knot 1: $36,418, Knot 2: $58,388, Knot 3: $73,611, and Knot 4: $103,813. The Wald p-value in **Table 4** for SES spline covariates (p = 0.031) indicated that median family income spline covariates contributed significantly to the univariate model. Also, the Wald test p-values between spline covariate 1 and spline covariate 2 (Wald test p = 0.094), and spline covariate 2 and spline covariate 3 (Wald test p = 0.118), approached significance, indicating that their slopes approached being significantly different from each other. Spline covariate 1 and spline covariate 3 were found to be statistically significantly different from each other (Wald test p-value = 0.029). Therefore there is a non-linear relationship between median family income and non-dominant tibial SOS. Furthermore, the R^2 for the spline covariates association with non-dominant tibial SOS was 3.01%, which was nearly double that of the R^2 for the linear association between median family income and non-dominant tibial SOS (R^2 = 1.76%). This further indicated the variance in non-dominant tibial SOS was explained better with the use of splines.

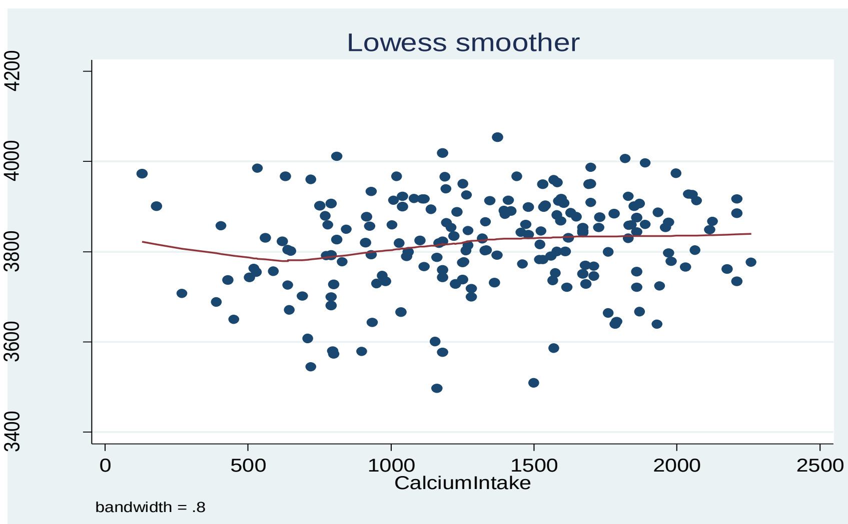


Figure 8: LOWESS curve for calcium intake and non-dominant tibial SOS.

In **Figure 8**, the relationship between calcium intake and non-dominant tibial SOS was found to be linear however with minor fluctuations. Creating restricted cubic splines was not required for analysis.

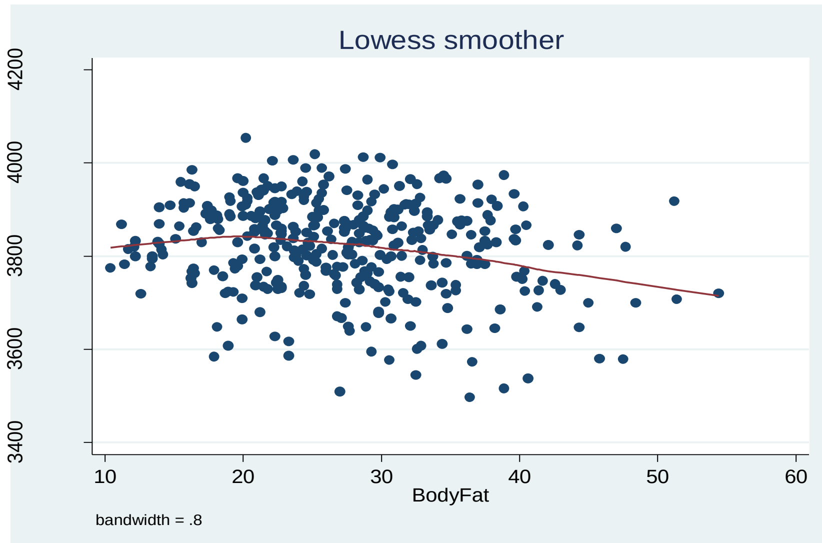


Figure 9: LOWESS curve for between % body fat and non-dominant tibial bone SOS

A non-linear relationship can be observed in **Figure 9** between percentage body fat and non-dominant tibial bone SOS. Therefore linear splines were created for the explanatory variable percentage body fat. Non-dominant tibial SOS appears to increase with percent body fat until 20%, and thereafter decrease, thus a knot location was specified at 20 % body fat, creating two splines. The Wald test p-value between body fat percentage spline covariates was p = 0.008, indicating that their slopes are statistically significantly different from each other. This confirmed a non-linear relationship between percentage body fat and non-dominant tibial SOS.

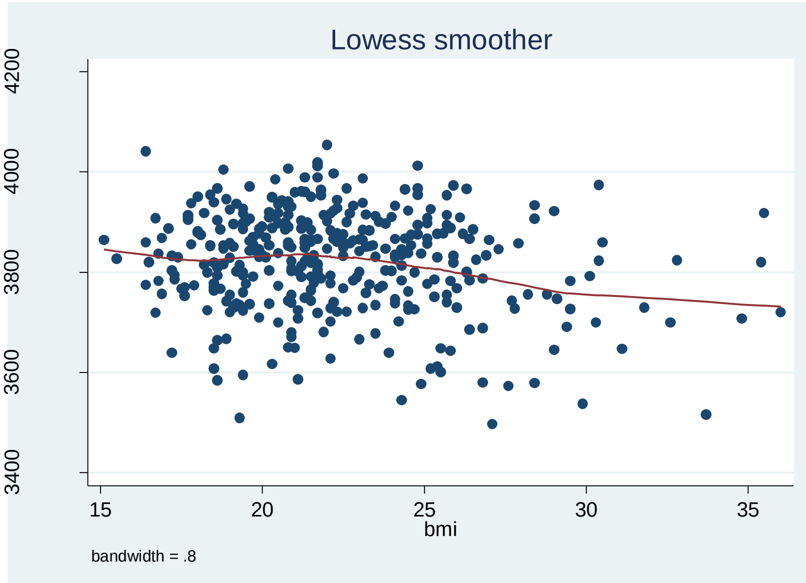


Figure 10: LOWESS curve for between BMI and non-dominant tibial bone SOS.

A non-linear relationship was observed in **Figure 10** between BMI and non-dominant tibial bone SOS. Therefore linear splines were created for the explanatory variable BMI. A knot location was specified at 22 kg/m^2 creating two splines. The Wald test p-value between BMI spline covariates (p = 0.02) indicated that BMI spline covariates were significantly different from each other, confirming there is a non-linear relationship between BMI and non-dominant tibial SOS.

Additional SES variables were explored for their association with non-dominant tibial SOS and some were assessed for linearity, such as employment rate and prevalence of low income. These variables were found to exhibit linear associations with non-dominant tibial SOS however were deemed less important predictors compared to median family income, and since only one indicator will be used to reduce multiple comparisons, those variables will not be analyzed further.

4.3 Unadjusted regression

Univariate associations are evaluated in this subsection. Univariate associations were examined between distal predictors SES and school with non-dominant tibial SOS, and between proximal (intermediate) factors, with non-dominant tibial SOS. Associations between SES and school with proximal risk predictors to non-dominant tibial SOS (or intermediate factors) were also examined. Univariate analysis examining calcium intake utilized the **mi estimate: regression** command in STATA 12, in order to include the multiply-imputed values for the variable calcium intake. STATA provided the same R^2 for each imputed dataset for a given model therefore the R^2 reported reflected all ten imputed data sets, and reporting a mean and range of R^2 was unnecessary. Similarly, for LRT analyses that were run with the multiply-imputed calcium intake values in the model, only one LRT p–value was reported, as each imputed dataset generated the same p-value, and it was unnecessary to report the mean p-value and range of the p-value.

4.3.1 Univariate associations between distal (SES and school) with non-dominant tibial SOS (Hypotheses #1 and 2) and proximal predictors with the outcome non-dominant tibial SOS (Hypothesis # 3)

As observed in the univariate association results presented in **Table 4**, the SES distal explanatory factor median family income had a significant non-linear association with non-dominant tibial bone SOS, after accounting for the clustering of participants within schools. The b-coefficients of spline covariates are not interpretable separately. However, their significance can be evaluated as a group of covariates. The Wald p-value of 0.031, accounting for the clustering effect of participants within schools, indicated that the median family income spline covariates contributed significantly to the model, providing evidence that SES is a significant predictor of non-dominant tibial bone SOS. Median family income spline covariates accounted for 3.01% of the variance explained.

Table 4. Unadjusted linear regression coefficients for the relationship between SES variables and non-dominant tibial bone SOS (m/s), BONES 2007-2008, adjusted for clustering in schools (n = 368)

SES Variable	b-coefficient	Robust standard error	P-value	R^2	Wald P-value for SES splines
Median family income ($)*			0.031	0.030	0.031
Spline 1	-0.0019	0.0013	0.218		
Spline 2	0.0083	0.0072	0.300		
Spline 3	-0.0255	0.0241	0.388		

*Knot locations for median family income: $36,418, $58,388, $73,611, $103,813.

From the results presented in **Table 5**, age had a significant positive association with non-dominant tibial SOS (b = 27.31, S.E. = 4.82, p < 0.0001), and explained 7.9% of the variance in non-dominant tibial SOS. Percentage body fat explained 5.1% of the variance in non-dominant tibial SOS and was highly significant (p = 0.008) while BMI explained 5.2% of the variation in non-dominant tibial SOS (p = 0.02). Calcium intake was positively associated with non-dominant tibial SOS, however non-significant and with an identical robust standard error to its coefficient (b = 0.021, S.E. = 0.021, p = 0.381). Physical activity exhibited a similar positive association, however with a near identical standard error to its coefficient and non-significance (b = 0.265, S.E. = 0.264, p = 0.362). Reporting yes to regular alcohol consumption was significantly positively associated with non-dominant tibial SOS (p = 0.039) which was inconsistent with literature pertaining to adults. Reporting yes to regular smoking yielded a positive however non-significant association (b = 34.4, p = 0.158). Reporting yes to a family history of osteoporosis had a positive association with non-dominant tibial SOS, trending towards statistical significance (b = 38.36, S.E. = 16.41, p = 0.067). Associations between non-dominant tibial SOS with the variables pertaining to regular smoking and family history of osteoporosis were also inconsistent with the literature on adults.

Table 5. Unadjusted linear regression coefficients for the relationship between proximal risk factors and non-dominant tibial bone SOS (m/s), BONES 2007-2008, adjusted for clustering in schools

Variable	N	b-coefficient	Robust standard error	P-value	R^2	Wald P-value for splines
Age (years)	366	27.31	4.82	0.002	0.079	
BMI (kg/m^2)*	365				0.052	0.02
Spline 1		4.26	2.84	0.194		
Spline 2		-9.81	2.33	0.008		
Percent body fat†	362				0.051	0.008
Spline 1		4.01	0.949	0.008		
Spline 2		-3.39	0.947	0.016		
Calcium intake (mg)‡	378	0.021	0.021	0.381	0.005	
Physical activity§	348	0.265	0.264	0.362	0.005	
Regular alcohol consumption (yes vs. no)	366	31.91	5.58	0.002	0.016	
Regular smoking (yes vs. no)	366	34.30	20.68	0.158	0.01	
Family history of Osteoporosis (yes vs. no)	353	38.36	16.41	0.067	0.012	

Abbreviation: N, sample size.
*Knot for BMI was specified at 22 kg/m^2.
†Knot for % body fat was specified at 20%.
‡Calcium intake analysis utilized multiply-imputed dataset.
§Physical activity measured by Total Exercise Score.

When the univariate association between School and non-dominant tibial SOS was examined, as presented in **Table 6**, school explained 15.8 % of the variance in non-dominant tibial SOS and was significantly associated (p = 0.0001). There was a large increase in the R^2 compared to SES by over a 12% difference as seen in **Table 4**. BT school was used as the referent group. WC school was significantly different in non-dominant tibial SOS compared to BT school (p = 0.0001), with WC school yielding a parameter estimate of 105.07 m/s less than the non-dominant tibal SOS of the referent school.

Table 6. Unadjusted linear regression coefficients for the relationship between School and non-dominant tibial SOS (m/s), BONES 2007-2008 (n = 368)

Variable	**b-coefficient**	**Standard error**	**P-value**	**R^2**
School			**0.0001**	**0.158**
BT	(Referent)			
LS	4.66	22.81	0.838	
ND	-1.05	21.32	0.961	
SF	-33.19	22.76	0.146	
SM	-32.58	24.03	0.176	
WC	-105.07	22.31	0.0001	

4.3.2 Univariate associations between distal (SES and school) and proximal risk factors (intermediate risk factors) to non-dominant tibial SOS (Hypothesis # 4)

The results in **Table 7** assessed the association between proximal risk factors to non-dominant SOS and median family income, and are presented in two parts. In the top part of the table, the associations between median family income and proximal risk factors that were continuous dependent variables were assessed. Therefore linear regression coefficients were presented for age, BMI, percentage body fat, calcium intake and physical activity. The bottom part of the table assessed the association between median family income and proximal risk factors that were dichotomous dependent variables. Therefore odds ratios are presented for regular smoking, regular alcohol and family history of osteoporosis. From the univariate association results presented in **Table 7**, the proximal factors that were significantly associated with SES were BMI, calcium intake and regular smoking. SES was significantly negatively associated with BMI (b = -

0.14, p = 0.049) and significantly positively associated with calcium intake (b = 50.0, p = 0.037). Percentage body fat trended towards statistical significance (p = 0.088), and was observed to be negatively associated with SES (b = -0.50).

Regular alcohol consumption was not significantly associated with SES (OR = 0.97, 95% CI = 0.84 – 1.11), which is expected for this age group. SES was significantly associated with regular smoking, with a $10,000 increase in median family income exhibiting a protective effect against regular smoking (OR = 0.72, 95% CI = 0.64 – 0.81) and decreased the odds of reporting yes to regular smoking by 28% among adolescent females in this sample. Family history of osteoporosis was not significantly associated with SES (OR = 1.15, 95% CI = 0.99 – 1.34).

Table 7. Unadjusted linear regression coefficients, and odds ratios, for the relationships between SES (**median family income**) and the outcomes **proximal risk factors** to non-dominant tibial SOS (m/s) (intermediate factors to bone SOS), BONES 2007-2008 – **per $10,000**adjusted for clustering in schools

Dependent Variable	**N**	**b-coefficient**	**Robust standard error**	**P-value**	**R^2**
Age (years)	410	0. 007	0. 03	0.802	0.0002
BMI (kg/m^2)	408	-0. 14	0. 05	0.049	0.005
% Body fat	405	-0. 50	0. 20	0.088	0.012
Calcium intake (mg)*	391	50.0	20.0	0.037	0.048
Physical activity†	388	-0. 70	0. 80	0.467	0.002
Dependent Variable	**N**	**OR**		**95% CI**	**Pseudo -R^2**
Regular alcohol (yes)	410	0.97	0.066	0.84 – 1.11	0.0007
Regular smoking (yes)	410	0.72	0.043	0.64 – 0.81	0.0001
Family history of osteoporosis (yes)	395	1.15	0.088	0.99 – 1.34	0.062

Abbreviations: CI, confidence interval; n, number; OR, odds ratio.
*Calcium intake analysis utilized multiply-imputed dataset.
†Physical activity measured by Total Exercise Score.

T-tests were also conducted for sensitivity analysis in order to assess the consistency of the results of the dichotomous dependent variables, and evaluated if there was a significant difference in the means of median family income between the yes and no groups, for the dichotomous variables of regular alcohol consumption and regular smoking, as well as family history of osteoporosis (**Table 8)**. As displayed in **Table 8**, results for the *t*-tests indicated that there was not a significant difference in median family income between those who drink alcohol regularly versus those who do not (p = 0.59) which is consistent with the univariate logistic regression result in **Table 7**. The *t*-test result for regular smoking however revealed a significant difference in median family income between those who smoke regularly versus those who do not (p = 0.0003), also consistent with the univariate logistic regression result in **Table 7**. Compared to those who reported not smoking regularly, those who reported smoking regularly had a mean median family income in the DA that they live in of approximately $11,000 lower.

Table 8. *t*-test results for median family income by alcohol and smoking status and family history of osteoporosis, BONES 2007-2008

Variable	**n**	**Mean**	**SD**	***T***	**P-value**
Regular Alcohol				0.54	0.59
Yes	83	$67,123	$18,837		
No	327	$68,410	$19,541		
Regular Smoking				3.61	0.0003
Yes	44	$58,432	$15,962		
No	366	$69,417	$19,418		
Family History of Osteoporosis					
Yes	39	$73,185	$20,132	-1.69	0.09
No	356	$67,661	$19,296		

Abbreviations: n, sample size; SD, standard deviation.

As presented below, **Table 9** examined the association between School and proximal risk factors to non-dominant tibial SOS. Percentage body fat, calcium intake and physical activity were continuous dependent variables and are presented in the top row of the **Table 9,** and dichotomous dependent variables smoking, alcohol and family history of osteoporosis are presented in the bottom row.

Table 9. Unadjusted linear regression coefficients, and odds ratios, for the relationships between **School** and the outcomes **proximal risk factors** to non-dominant tibial SOS (m/s) (intermediate factors to bone SOS), BONES 2007-2008

	Dependent Variable								
Variable	**Percent body fat**			**Calcium intake (mg)***			**Physical activity†**		
	b-coefficient	**SE**	**P-value**	**b-coefficient**	**SE**	**P-value**	**b-coefficient**	**SE**	**P-value**
School									
BT	(Referent)			(Referent)			(Referent)		
LS	5.61	1.90	0.003	205.32	175.60	0.256	9.00	6.37	0.158
ND	0.763	1.79	0.670	246.06	178.01	0.184	2.56	6.03	0.671
SF	1.38	1.91	0.472	334.89	162.17	0.049	9.59	6.47	0.139
SM	1.51	2.03	0.459	68.02	205.54	0.745	14.49	6.86	0.035
WC	5.93	1.82	0.001	-149.28	158.77	0.357	11.26	6.13	0.067
N	**405**			**391***			**388**		
R^2	**0.084**			**0.104**			**0.027**		
P-value	**0.0001**			**0.0004**			**0.061**		

Variable	**Regular smoking (yes vs. no)**			**Regular Alcohol (yes vs. no)**			**Family history of osteoporosis (yes vs. no)**		
	OR	**SE**	**95% CI**	**OR**	**SE**	**95% CI**	**OR**	**SE**	**95% CI**
School									
BT	(Referent)			(Referent)			(Referent)		
LS	1.92	1.57	0.39 – 9.52	5.75	4.50	1.24 – 26.63	0.424	0.305	0.10 – 1.73
ND	1.39	1.10	0.29 – 6.60	3.93	3.02	0.87 – 17.72	0.625	0.392	0.18 – 2.14
SF	0.361	0.37	0.05 – 2.72	0.733	0.661	0.13 – 4.29	0.439	0.316	0.11 – 1.80
SM	1.41	1.24	0.25 – 7.88	1.38	1.21	0.25 – 7.68	0.375	0.304	0.08 – 1.84
WC	1.57	1.26	0.33 – 7.54	2.71	2.12	0.59 – 12.56	0.595	0.382	0.17 – 2.09
N	**410**			**410**			**395**		
Pseudo-R^2	**0.024**			**0.06**			**0.009**		
P-value	**0.248**			**0.0001**			**0.805**		

Abbreviations: CI, confidence interval; n, number; OR, odds ratio; SE, standard error. *Calcium intake analysis utilized multiply-imputed dataset.
†Physical activity measured by Total Exercise Score.

School was found to be significantly associated with percentage body fat (p = 0.0001) and calcium intake (0.0004), and trended towards a significant association with physical activity (p = 0.061). School attended was also found to be significantly associated with alcohol consumption (p = 0.0001). School attended however exhibited a non-significant association with smoking (0.248) and family history of osteoporosis (p = 0.805).

4.4 Multivariable regression models

4.4.1 Multivariable linear regression models (Hypotheses # 1 and 2)

Multivariable regression models were initially adjusted for covariates and confounders based on *a priori* reasoning as seen in **Tables 10a and 10b**. Multivariable regression examining proximal risk factors with non-dominant tibial SOS were examined in **Table 11**. Model selection to develop a parsimonious model was carried out by backwards elimination, as presented in **Tables 12a and 12b**. Variables with a Wald or LRT *p*-value of > 0.2 were eliminated. Therefore the variables in the following models were included based on "intelligent" model building after *a priori* reasoning. Multivariable logistic regression analysis examined the probability of low vs. high bone SOS as seen in **Tables 15a** and **15b.** Clustering of participants within schools was also taken into account by use of the **cluster(School)** command in STATA. An adjusted-R^2 was unavailable when accounting for the clustering effect therefore the R^2 value was reported for models accounting for the clustering effect.

In **Table 10a**, Model 1 was fully adjusted for age, percent body fat, participants' family history of osteoporosis, calcium intake, physical activity, regular smoking and alcohol consumption and also accounted for the clustering of participants within schools. SES splines were found to be trending towards significance (Wald p-value = 0.06).

Table 10a. Multivariable linear regression model for non-dominant tibial SOS (m/s), BONES 2007-2008 – Full model, adjusted for clustering in schools (n = 374)

	Model 1		
Variable	**b-coefficient**	**Robust standard error**	**P-value**
Age (years)	28.76	5.62	0.009
Percent body fat			
Spline 1	-0.679	1.32	0.647
Spline 2	-3.33	0.714	0.029
Family History of Osteoporosis (yes vs. no)	24.45	11.51	0.122
Calcium Intake (mg)†	0.01	0.016	0.601
Physical Activity	0.164	0.150	0.353
Regular Smoking (yes vs. no)	28.87	20.13	0.231
Regular Alcohol (yes vs. no)	-0.079	7.96	0.993
Median family income ($)*			
Spline 1	-0.002	0.001	0.154
Spline 2	0.01	0.006	0.208
Spline 3	-0.032	0.002	0.230
R^2	**0.193**		
Model P-value	**0.0001**		
Wald P-value for % body fat splines	**0.005**		
Wald P-value for SES splines	**0.06**		

*Knot location for median family income: $36,418, $58,486, $73,576, $103,664.
†Multiply-imputed dataset utilized as calcium intake was included in this model. An adjusted-R^2 was not provided when accounting for clustering, therefore an R^2 was reported (and for one multiply-imputed dataset). Wald P-values reported for one multiply-imputed dataset.

As seen in **Table 10b**, in the fully adjusted fixed effects model (Model 2), after adjusting for age, percent body fat, family history of osteoporosis, calcium intake, physical activity, regular smoking and alcohol consumption, school was found to contribute significantly to the model (LRT p-value = 0.0001), however SES was non-significant (LRT p-value = 0.208).

Table 10b. Multivariable linear regression model for non-dominant tibial SOS (m/s), BONES 2007-2008 – Full model including school (fixed effects) (n = 374)

	Model 2		
Variable	**b-coefficient**	**Standard error**	**P-value**
Age (years)	27.41	4.95	0.0001
Percent body fat			
Spline 1	-1.12	2.97	0.706
Spline 2	-2.37	0.830	0.005
Family History of Osteoporosis (yes vs. no)	30.13	15.85	0.058
Calcium Intake (mg) †	-0.007	0.014	0.609
Physical Activity	0.249	0.183	0.175
Regular Smoking (yes vs. no)	23.16	16.64	0.165
Regular Alcohol (yes vs. no)	0.989	13.06	0.940
Median family income ($)*			
Spline 1	-0.002	0.001	0.034
Spline 2	0.008	0.004	0.036
Spline 3	-0.025	0.013	0.052
School			
BT	(Referent)		
LS	-2.25	23.37	0.924
ND	-6.17	20.71	0.766
SF	-21.21	22.68	0.350
SM	-6.56	24.54	0.789
WC	-98.46	22.76	0.0001
Adj-R^2	**0.293**		
Model P-value	**0.0001**		
LRT P-value for % body fat splines	**0.0001**		
LRT P-value for SES splines	**0.208**		
LRT P-value for school	**0.0001**		

*Knot location for median family income: $36,418, $58,486, $73,576, $103,664.
†Multiply-imputed dataset utilized as calcium intake was included in this model. LRT P-values reported for one multiply-imputed dataset.

It should be noted that the fully adjusted models included variables that were exhibiting coefficients in opposite directions to that of established literature, such as the variables family history of osteoporosis and regular smoking, therefore these fully adjusted models (Model 1 and 2) were considered interim models. However, an important conclusion to observe is that despite the adjustment of all the variables, school still contributed significantly to the model, and appeared to override the significance of SES, suggesting an important impact of school on non-dominant tibial SOS.

Post-estimation testing for assumptions of linear regression was conducted for the all models as seen in **Table 14**. The graphs for the post-estimation tests of the assumptions for Model 2 are shown below. The fully-adjusted fixed effects Model 2 was tested for normality of errors as seen below in **Figure 11**, and found the residuals to be normally distributed therefore not violating the assumption of the normality of errors as skewness was minimal.

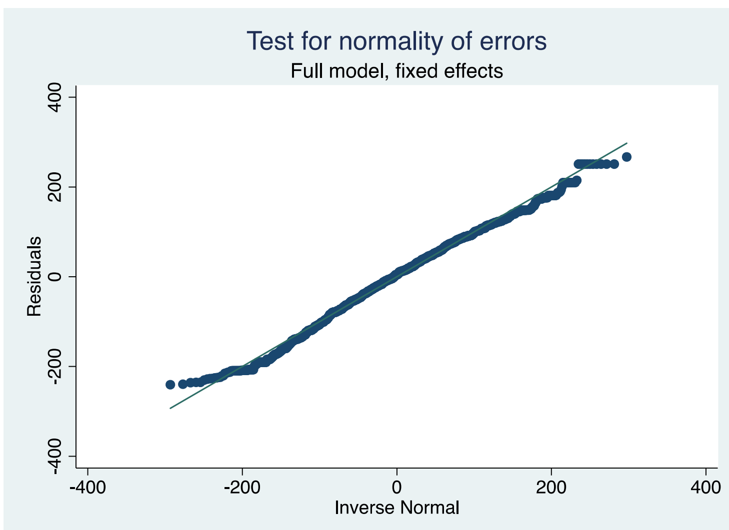


Figure 11 – Testing for normality of errors for Model 2, BONES 2007-2008

Note: Results of one multiply-imputed dataset

Model 2 was tested for homoskedasticity as seen below in **Figure 12**, and was observed not to be severely heteroskedastic therefore met the assumption for linear regression.

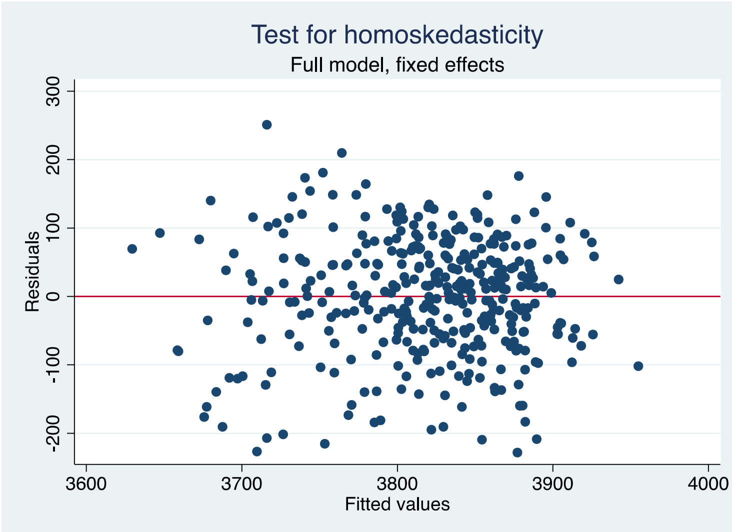


Figure 12 – Testing for homoskedasticity for Model 2, BONES 2007-2008

Note: Results of one multiply-imputed dataset

In **Table 11**, the proximal risk factors to non-dominant tibial SOS were analyzed in a multivariable linear regression model for their association with non-dominant tibial SOS. As some of these variables were thought to fall in the causal pathway between SES and bone SOS, they were analyzed separately, and consequently excluded from the final model. As seen in **Table 11**, the model was found to trend towards significant (p = 0.072), with age remaining a significant predictor of non-dominant tibial SOS (b = 30.24, Robust S.E. = 4.71, p = 0.004). Percentage body fat splines were also found to contribute significantly to the model. Calcium intake (b = 0.013, p = 0.505) and physical activity (b = 0.118, p = 0.548) exhibited a positive however non-significant association with non-dominant tibial SOS. In addition to calcium intake and physical activity exhibiting p-values greater than 0.2, since percentage body fat, calcium intake, and physical activity were thought to be in the causal pathway between SES and bone SOS, they were not included in the final model.

Table 11. Multivariable linear regression model between proximal risk factors to non-dominant tibial SOS (intermediate risk factors) and non-dominant tibial SOS (m/s), BONES 2007-2008, adjusted for clustering in schools (n = 378)

	Model 3		
Variable	**b-coefficient**	**Robust standard error**	**P-value**
Age (years)	30.24	4.71	0.004
Percent body fat			
Spline 1	0.621	1.44	0.694
Spline 2	-3.67	0.838	0.030
Calcium intake (mg)*	0.013	0.017	0.505
Physical activity	0.118	0.177	0.548
R^2	**0.155**		
Model P-value	**0.072**		

* Multiply-imputed dataset utilized as calcium intake was included in this model. An adjusted-R^2 was not provided when accounting for clustering, therefore an R^2 was reported (and for one multiply-imputed dataset).

After backwards elimination of variables with a p-value greater than 0.2 in a full model and based on reasoning of excluding variables that were exhibiting associations inconsistent with literature, the following models were built. These models included age, and the main predictors SES and school, as presented in **Table 12a** and **Table 12b.**

Table 12a. Multivariable linear regression model for non-dominant tibial SOS (m/s), BONES 2007-2008 – Final model, adjusted for clustering in schools (n = 366)

	Model 4		
Variable	**b-coefficient**	**Robust standard error**	**P-value**
Age (years)	27.05	5.16	0.003
Median family income ($)*			
Spline 1	-0.002	0.001	0.293
Spline 2	0.008	0.007	0.326
Spline 3	-0.025	0.024	0.345
R^2	**0.106**		
Model P-value	**0.003**		
Wald P-value for SES splines	**0.361**		

*Knot location for median family income: $36,418, $58,387, $73,610, $103,812. An adjusted-$R^2$ was not provided when accounting for clustering, therefore an R^2 was reported.

Assessing the association between SES and non-dominant tibial SOS, after accounting for the clustering of participants in schools as seen in Model 4 in **Table 12a**, SES spline covariates were found to be non-significant (p-value = 0.361).

Table 12b shows the fixed effects model, Model 5, with school included in the model. School was a significant predictor of non-dominant tibial SOS (LRT p-value = 0.0001), however similar to results presented in **Table 10b** in the full model, SES was non-significant (LRT p-value = 0.317). The R^2 in Model 5 also increased by 11.4% compared to the R^2 in Model 4 when school is included in the model, with Model 5 explaining 22% of the variance in non-dominant tibial SOS.

Table 12b. Multivariable linear regression model for non-dominant tibial SOS (m/s), BONES 2007-2008 – Final model excluding variables with p > 0.2; Model including school (fixed effects) (n = 366)

	Model 5		
Variable	**b-coefficient**	**Standard error**	**P-value**
Age (years)	28.06	4.84	0.0001
Median family income ($)*			
Spline 1	-0.002	0.001	0.069
Spline 2	0.006	0.004	0.097
Spline 3	-0.018	0.012	0.137
School			
BT			
LS	-16.59	22.84	0.468
ND	-9.07	20.45	0.658
SF	-30.60	22.11	0.167
SM	-12.05	24.31	0.620
WC	-110.94	22.67	0.0001
Adj-R^2	**0.220**		
Model P-value	**0.0001**		
LRT P-value for SES splines	**0.317**		
LRT P-value for School	**0.0001**		

*Knot location for median family income: $36,418, $58,387, $73,610, $103,812.

The significance of school was observed to be persistent in the fixed effects models that included both median family income and school, as seen in **Tables 10b** and **12b.** As it was determined that school SES and student SES were not strongly collinear, these results indicate that school plays a significant role in a student's non-dominant tibial bone SOS.

In Models 4 and 5, the inclusion of age in the models may not have been necessary due to age likely not being a confounder in this adolescent age group, as during the teenage years SES is not associated with age (p-value = 0.802). For these models, age was considered an adjustment covariate. A model adjusted for only the two main predictors showed that once school was introduced into the model with SES (Model 6), it overrode the effect of SES compared to univariate analysis without school in the model, as seen in **Table 13.**

Table 13. Multivariable linear regression models for non-dominant tibial SOS (m/s), BONES 2007-2008 - Alternative model excluding variables with p > 0.2 and age; Model including school (fixed effects) (n = 368)

	Model 6		
Variable	**b-coefficient**	**Standard error**	**P-value**
Median family income ($)*			
Spline 1	-0.002	0.001	0.082
Spline 2	0.006	0.004	0.139
Spline 3	-0.015	0.013	0.216
School			
BT	(referent)	--	--
LS	8.57	23.50	0.716
ND	-1.67	21.39	0.938
SF	-32.94	23.10	0.155
SM	-26.86	25.28	0.289
WC	-102.3	23.70	0.001
Adj-R^2	**0.148**		
Model P-value	**0.0001**		
LRT P-value for SES splines	**0.277**		
LRT P-value for School	**0.0001**		

*Knot location for median family income: $36,418, $58,387, $73,610, $103,812.

With school in the model, SES splines were no longer significant (LRT p-value = 0.277), due to the overriding effect of school over SES on non-dominant tibial SOS (LRT p-value = 0.0001). The R^2 in Model 6 increased by 11.8% compared to univariate model once school was included in the model.

4.4.2 Examination of interactions

Potential interactions were evaluated with no evidence of interactions. For example, the interaction between SES and age was examined. The interaction term was found to be non-significant and therefore age did not modify the effect of SES on non-dominant tibial SOS (LRT for interaction *p*-value = 0.638) and the interaction did not predict non-dominant tibial SOS. Further analyses of interactions were deemed unnecessary for this study.

4.4.3 Post-estimation tests for assumptions for linear regression

The assumptions of the normality of residuals and homoskedasticity were tested post-estimation for multivariable regression models. As presented in **Table 14**, the assumption of normality of residuals was met for Model 2, Model 4, Model 5 and Model 6. The assumption of homoskedasticity was met for Model 1, Model 3 and Model 4.

Table 14. Post-estimation tests of assumptions of multivariable linear regression models (assumption met vs. violated)

Model	Test for normality of residuals	Test for homoskedasticity
Model 1	Violated	Met
Model 2	Met	Violated
Model 3	Violated	Met
Model 4	Met	Met
Model 5	Met	Violated
Model 6	Met	Violated

Note: Test of normality of residuals based on **qnorm** graph. Test of homoskedasticity based on **rvfplot** graph (for models accounting for clustering) and **estat hettest** command.

For those models whose assumptions for linear regression were violated, they were not extreme violations however the results should be interpreted with caution.

4.4.4 Multivariable logistic regression model

As presented in **Table 15a** and **Table 15b**, the multiple logistic regression models evaluated the odds of having low bone SOS vs. high bone SOS using the final model, for the model assessing the effect of SES while accounting for clustering of participants in schools (Model 7), and the fixed effects model including school (Model 8). It should be noted that odds ratios for spline covariates are not interpretable without transformation, but can be assessed for their contribution to the model.

As observed in **Table 15a** (Model 7), after accounting for clustering and adjusting for age, SES spline covariates contributed to the model with a p-value of less than 0.2 (p = 0.181). Age continued to be a significant predictor of bone SOS, with a one year increase in age decreasing the odds of low bone SOS by 43% after adjusting for SES and clustering (OR = 0.57; 95% CI = 0.454 – 0.720).

Table 15a. Multivariable logistic regression analysis, BONES, 2007-2008; Predicting low bone SOS vs. high bone SOS for non-dominant tibial bone– Final model, adjusted for clustering in schools (n = 410)

		Model 7	
Variable	**OR**	**Robust Standard Error**	**95% CI**
Age (years)	0.57	0.067	0.454 – 0.720
Median family income*			
Spline 1	1.00002	0.00002	0.999993 – 1.00005
Spline 2	0.9999	0.00008	0.9997 – 1.00003
Spline 3	1.0004	0.0003	0.9999 –1.0009
Pseudo R^2	**0.063**		
Model P –value	**0.0001**		
Hosmer-Lemeshow goodness of fit test	**0.465**		
Wald P- value for SES splines	**0.181**		

Abbreviations: CI, confidence interval; OR, odds ratio.
*Knot location for median family income: $36,418, $58,387, $73,610, $103,812.

In **Table 15b**, in the fixed effects model (Model 8), school was found to have a significant contribution to the model (LRT p = 0.002), similar to results from the multivariable linear regression fixed effects models. SES spline covariates were non-significant (p = 0.807) with the inclusion of school in the model, appearing to override the significance of SES. The Hosmer-Lemeshow goodness-of-fit test p-value of Model 8 (p = 0.361) also indicated that the model is well-calibrated and the model's fit of the data was acceptable.

Table 15b. Multivariable logistic regression analysis, BONES, 2007-2008; Predicting low bone SOS vs. high bone SOS for non-dominant tibial bone – Final model excluding variables with p > 0.2; Model including school (fixed effects) (n = 410)

Model 8			
Variable	**OR**	**Standard error**	**95% CI**
Age (years)	0.554	0.081	0.416 – 0.737
Median family income ($)*			
Spline 1	1.00002	0.00003	0.99997 – 1.0001
Spline 2	0.9999	0.0001	0.9997 – 1.0001
Spline 3	1.0002	0.0003	0.9996 – 1.0008
School			
BT	(referent)		
LS	2.14	1.59	0.50 – 9.20
ND	1.06	0.736	0.274 – 4.13
SF	2.21	1.55	0.559 – 8.74
SM	1.84	1.38	0.424 – 7.98
WC	5.00	3.50	1.27 – 19.74
Pseudo-R^2	**0.105**		
Model P-value	**0.0001**		
Hosmer-Lemeshow goodness of fit test	**0.361**		
LRT P-value for SES splines	**0.807**		
LRT P-value for School	**0.002**		

Abbreviations: CI, confidence interval; OR, odds ratio.
*Knot location for median family income: $36,418, $58,387, $73,610, $103,812.

The effect estimates for the prediction of low bone SOS versus high bone SOS displayed in **Tables 15a** and **15b** generally reflected th results of the multiple linear regression models in **Tables 12a and 12b.** However, SES splines in Model 7 of **Table 15a** approached significance (LRT p-value = 0.181) compared to Model 4 in **Table 12a** (LRT p-value = 0.361). School remained consistently significant (LRT p-value = 0.002) in Model 8 similar to Models 2, 5 and 6, and appeared to override the effect of SES (LRT p-value = 0.807).

Although SES was non-significant when accounting for clustering in the multivariable linear and logistic regression models, SES still approached significance, and the importance of SES cannot be ignored. Also, although age was included in the model, it is likely not a confounder in the adolescent age group, as SES is not associated with age in this age group ($p = 0.802$). Since age did not fully meet the requirements to be a confounder, conclusions can be drawn from univariate analyses.

Reasons for the importance of SES and the explanations for the possible weak associations observed are discussed in Chapter 5. School however was found to be a significant predictor of non-dominant tibial SOS, as seen in all fixed effects models (Model 2, Model 5, Model 6 and Model 8), and these results indicated that there is a significant school effect.

CHAPTER 5: DISCUSSION & CONCLUSIONS

5.1 Pertinent Study Findings

Pertinent findings from this investigation are that:

1.) As seen in univariate analysis after accounting for the clustering effect, SES had a significant non-linear effect on non-dominant tibial SOS (Wald p = 0.031) in adolescent females from the Niagara Region and the City of Hamilton.

2.) As seen in univariate and multivariable analyses, school had a significant impact on the non-dominant tibial bone SOS (LRT p = 0.0001) in adolescent females from the Niagara Region and the City of Hamilton, which overrode and explained away the SES effect.

3.) Median family income was significantly non-linearly associated with non-dominant tibial bone SOS. A negative association was exhibited up to approximately $58,387, with adolescents from lower middle-class median family incomes having the lowest bone SOS. Bone SOS exhibited a positive association with median family income after $58,387.

5.2 Relation to previous research

5.2.1 Sample characteristics

The average median family income of this study sample of $68,162 reflected the 2008 median family income of Ontario of $70,910, and of Canada of $68,860 (Statistics Canada, 2011). The average prevalence of families below the low-income after-tax income was 9.4 % (SD 10.2), which reflects the prevalence of families below the low-income after-tax income in Canada which was 9.4 % in 2008 (Statistics Canada, 2011). Therefore in terms of SES, it appears that this sample is reflective of the Canadian population. The average age of this sample study was 15.7 (SD 1.0) years with a range of 14-20 years which provided an appropriate age distribution to investigate bone health as peak bone mass is accrued by approximately age 20 years. The sample examined was found to have participants with a mean daily calcium intake of 1306.6 (SD 469.4) mg. This level of intake falls within the daily recommended intake of 1200 - 1500 mg however was high compared to values typical for calcium intake among adolescent females in Canada. As seen in a Canadian study of 785 adolescents in Vancouver, British Columbia, the mean calcium intake for female adolescents was 815 (SD 26) mg per day (Barr, 1994), which is lower than the daily recommended intake. However another study in the U.S. studied 3307 children and adolescents in 48 states, examining the food intakes of youth compared with recommendations, and found that the mean number of servings were below recommendations for all the food groups except the dairy group (Munoz, Krebs-Smith, Ballard-Barbash, & Cleveland, 1997). Despite the findings by the U.S. study, the levels of daily calcium intake in our sample study were higher than expected for Canadian adolescent females. Potential reasons for the inflated level of calcium intake in our study are discussed further in this chapter. Physical activity was also higher than expected, likely due to the sampling of students from physical education classes. Future studies should sample students from a random sample of classes.

5.2.2 Associations between distal factors (SES and school) and non-dominant tibial SOS (Hypotheses #1 and 2)

Findings from this study demonstrated that there is a significant non-linear relationship between median family income and non-dominant tibial SOS, in univariate analysis after accounting for clustering of participants within schools (Wald $p = 0.031$). As seen in the LOWESS curve of **Figure 7**, the wide V-shape of the curve indicated that the probability of higher non-dominant tibial SOS increased with an increase in median family income after approximately $58,000. However, below $58,000, the probability of higher non-dominant tibial SOS increased with a decline in median family income, with the probability of better bone SOS higher for those from a very low median family income. This non-linear relationship between SES and bone health is a new finding unique to this study. This result could have been artifact due to random variation, however since it is statistically significant it is likely not random, and is possibly real in this sample. It is also possible that this finding is spurious. However there can also be several explanations for this result, but these are only suggestions. One may be that those adolescents from lower median family incomes having been required to take on part-time jobs, which likely involved walking and standing and therefore weight-bearing activity, however this is just one possible explanation. Another possibility is that these students may also have had less access to vehicles therefore walked more often, which is another speculation. This probability of higher bone SOS decreased with increased median family income up to $58,000, considered the lower-middle class. Lower-middle class ranges in family income from $40,000 – 60,000 in Canada (Hennessy, 2013). However, the positive association between median family income and non-dominant tibial SOS after approximately $58,000 is somewhat substantiated with previous literature on the positive association between SES and bone health. Previous studies have predominantly demonstrated a linear positive relationship between SES and bone health. For example, Alver and colleagues (2007) investigated 702 women in Oslo, Norway, over the age of 40, and colleagues found that age-adjusted mean BMD in women living in a less affluent Eastern region were significantly lower in BMD than the Western region (OR = 1.87, 95% CI: 1.22 – 2.87, of low BMD in women in Eastern region versus West) (Alver, Sogaard, Falch, & Meyer, 2007). However, Brennan and colleagues (2009) studied 1494 Australian adult women and found that there was a somewhat non-linear relationship, with lowest and highest quintiles of SES having the lowest BMD as measured by DXA. Since most studies on SES and bone health focused on adult women in other countries, our study is a novel study of this age group in Canada, and the non-linear relationship may be real. However, a recent study in Spain, investigating SES and bone mass in 322 Spanish adolescents found no association between SES (family affluence scale, parental education, and occupation) and bone mass (Gracia-Marco et al., 2012). This study looked at both boys and girls together however, and used different SES indicators. One more study by Crandall and colleagues in Los Angeles, USA, studied 729 adults aged between 25-75 years, and found that childhood advantage score and adult education level were positively associated with lumbar spine BMD after adjustment for race, study site, body weight, menopausal status, and age (Crandall et al., 2012). Although the Crandall study demonstrated a linear relationship, they noted that childhood SES factors may influence the acquisition of bone mass, especially trabecular bone mass, during the growing years (Crandall, et al., 2012). It appears that previous studies did not further examine potential non-linear relationships, by modeling using restricted cubic splines or any transformation, rendering our study to be novel.

Therefore SES plays an important role on bone SOS, as seen in the significant association in univariate analysis after accounting for clustering. However, this association was weak. The weaker association may be due to the smaller variation of SES and bone SOS in Canada, as well as in this Canadian sample, compared to other countries that investigated SES and bone health. Another reason for the weaker association may be attributed to the use of aggregate-area level SES. Although it was necessary to use aggregate-area level SES, as data for individual-level SES was inaccurate or unavailable, assigning group-level SES to an individual likely reduced the variation of SES in the sample. Due to the above reasons, this sample had a smaller range of SES compared to other countries, rendering the results less pronounced. However, it can still be concluded that SES is an important predictor of bone health.

With a median family income in Canada of $68,860, Canada is generally a prosperous nation. This Canadian sample similarly appeared to be prosperous compared to other international study samples and populations. The average median family income of our study sample reflected the median family income of the population of Canada, with the range of median family income of this sample spanning $93,321, from $27,933 to $121,254. The SES gradient in Canada is not quite as wide as other nations, and Canada has a low income inequality, leading to less health inequality (Public Health Agency of Canada, 2012), compared to other nations. The income equality index is measured by the Gini coefficient, with values ranging from 0 to 1, and indicates national income distribution. The value of 0 indicates perfect income equality and 1 indicates perfect income inequality (Organisation for Economic Co-operation and Development, 2011). Canada has a Gini coefficient of 0.32 which is better than the income equality of other nations that investigated SES and bone health in children, such as Lebanon, whose Gini coefficient is 0.36 (Organisation for Economic Co-operation and Development, 2011). There could also be a smaller variation in bone SOS, as Canada is generally a healthy nation. This sample appeared to be a healthier sample, with values for non-dominant tibial SOS exhibiting a slightly negative-skewed distribution, such that there were a larger number of higher values. Canada has a shallow socioeconomic gradient in health status (smaller disparities in health inequality compared to countries with sharp social and economic differences). Countries which have shallow gradients (greater income equality) tend to have healthier societies (Public Health Agency of Canada, 2012). Also, although low SES increases the likelihood of developing poor health outcomes, many young people still develop into healthy adults (Public Health Agency of Canada, 2012). Therefore, despite an arguably weak association in univariate analyses after accounting for clustering, SES was still found to have a significant effect on bone health among adolescent females in Canada.

Therefore, the findings from this study generally substantiate previous literature focusing on bone health and SES (Akarirmak, 1996; Arabi, et al., 2004; Brennan S. L, 2009; Brennan, et al., 2010; M. C. Wang & Dixon, 2006), that SES is generally positively associated with bone health (from lower middle-class SES onwards). However, those previous studies had numerous methodological differences and limitations. Those limitations included small sample sizes and low external generalizability to the Canadian and U.S. populations as well as the adolescent female population. For example, Brennan and colleagues examined the association between SES and BMD in 1494 adult women in Australia, suggesting a positive association between SES and bone health; however this study examined adult women, ignoring the adolescent population, and utilized BMD as the outcome measure versus the measure of bone SOS, which captures more information about bone health (Brennan S. L, 2009). The study by Akarirmak investigating SES and bone SOS in 60 school children in Turkey had a low study power due to its small sample size, as well as low generalizability to a Canadian female adolescent population (Akarirmak, 1996).

This current study focused on Canadian adolescent females, a population often ignored in the literature on bone health and SES. This study also measured bone quality by use of the QUS measuring bone speed of sound versus the DXA device which measures bone mineral density. Also unique to this study was the use of AABCD estimators of SES, which were utilized to obtain SES data for all individuals. The influence of area-level measures of SES among the Canadian adolescent population had been previously investigated by the Canadian researchers Janssen and colleagues. However that study examined obesity as the outcome. Additionally, although the findings of our study remained consistent with previous literature of SES being generally significantly positively associated with bone health, our study differed from previous studies as it furthermore found that school also plays a critical role in the bone health of adolescent females in Canada. Likelihood ratio tests revealed that school ($p = 0.0001$), age ($p = 0.0001$), and percentage body fat ($p = 0.0001$) were significant contributors to non-dominant tibial SOS. This was consistent with literature for age and percentage body fat, however observing the importance of school is unique to this study. In the fixed effects model, when school was introduced into the model, it appeared to override the effect of SES on the participants' non-dominant tibial SOS (LRT p-value for school = 0.0001).

This is a cross-sectional study therefore causality cannot be conclusively determined. However, potential mechanisms linking SES, schools and bone health among adolescent females can be postulated, which could facilitate the elucidation of the causal pathway between SES, schools and bone health for future investigations. Since this study focused on adolescents in Canada, and is a novel study investigating SES, school and bone SOS during the critical youth period in a Canadian sample, it is a starting point for the investigation of the relationship between SES, school, and bone SOS among children and adolescents in Canada and the U.S.

5.2.2.1 Investigation of SES and bone health (Hypothesis #1)

SES plays a role in health outcomes and affects the likelihood of bone health outcomes as initially hypothesized, although not in every case, as some exposed to lower SES circumstances still develop good health outcomes. However, it is beneficial to explore pathways from SES to bone health outcomes in Canada. Psychosocial factors could play a role with SES including social support and attitudes towards physical activity such as sports, and consumption of foods such as dairy products. Higher SES is generally linked to greater health knowledge in addition to a greater income, allowing for healthier lifestyle choices including healthier food choices that can be afforded by families of higher SES (James, Nelson, Ralph, & Leather, 1997), including dairy products which improve bone health and peak bone mass (Cashman, 2002).

In our current study, those with lower SES had lower calcium intake. Studies that have documented fast food and commercial foods indicate that these foods contain more fat and hold less nutritious value. Families with lower SES may be more likely to purchase fast food than cooking healthy foods, as it is cheaper and more accessible (James, et al., 1997). As well there is a higher concentration of fast food restaurants in areas of lower SES (Hanson & Chen, 2007), which could lead to a lack of nutrients necessary in a healthy diet for bone health, such as calcium intake (Janssen, Boyce, Simpson, & Pickett, 2006). For example, the consumption of milk and citrus beverages might be substituted by the consumption of soft drinks. Healthier diets including foods such as fruits and vegetables usually tend to be pricier than diets based on foods with either or both added sugars and fats (Janssen, et al., 2006). One study in Australia examining the association between area-level SES and fast food purchasing found results that indicated that living in an area of a greater level of socioeconomic disadvantage increased an individual's odds of frequent fast food purchasing (Thornton, Bentley, & Kavanagh, 2011). This leads to a higher percentage of consumed calories from fat (Hejazi & Mazloom, 2009). A higher percentage of consumed calories from fat usually results in a lower percentage of consumed calories from healthier foods such as vegetables and dairy products necessary for healthy bones.

In a study conducted in Europe, Hoglund and colleagues examined the food habits of 1280 Swedish adolescents in relation to SES. They found that adolescents from areas of high SES ate breakfast more often compared to adolescents from areas with low SES. Girls in particular exhibited irregular meal patterns and unhealthy snack consumption such as the consumption of soft drinks, ice cream and potato crisps (Hoglund, Samuelson, & Mark, 1998). Families from a lower SES background are also likely to be less active, and have lower vitamin D intake (Hanson & Chen, 2007). Janssen et al (2006) also suggested that adolescents from families with lower SES may have less opportunity to participate in physical activity programs such as sports due to costs and poor parental support. The American Academy of Pediatrics (AAP) has recommended that older children and adolescents not meeting their daily 400 IU requirements of vitamin D should take a 400 IU/per day supplement (NIH, 2011). Students of higher SES may have the resources available and health knowledge necessary in order to expose themselves to adequate sunlight and invest in vitamin D supplements. Finally, neighbourhoods with lower SES tend to have fewer parks, and less safe streets (Janssen, et al., 2006), which may lead to lower levels of physical activity, and thus lower levels of bone health among other health problems.

5.2.2.2 Associations between SES and proximal factors to non-dominant tibial SOS (Hypothesis #4)

Findings from our study were similarly in accordance with Janssen and colleagues in finding that SES was significantly positively associated with calcium intake ($p = 0.037$) among Canadian adolescents in univariate analysis. As noted in the discussion above, the diet of lower socioeconomic groups tend to be lower in essential nutrients such as calcium (James, et al., 1997). Milk and milk products can be considered expensive and the adequate amounts necessary for optimal peak bone mass may not be affordable for families with a low family income. The Canadian cross-sectional analysis by Janssen and colleagues in 2006 examined the influence of individual and area-level measures of SES on obesity, unhealthy eating, and physical activity among 6684 Canadian adolescents and found that the odds for unhealthy eating increased for those living in an area with a low percentage of residents with a high school education, and the odds for physical inactivity increased with decreasing levels of material wealth and perception of family wealth (Janssen, et al., 2006). These unhealthy patterns associated with low SES not only affect the risk for obesity but also affect the risk for poor bone health and future risk for osteoporosis.

In addition, BMI was significantly negatively associated with SES ($p = 0.049$) and percentage body fat trended towards being significantly negatively associated with SES ($p = 0.088$). Therefore an increase in SES is associated with a decrease in percentage body fat and BMI and thus possibly improved bone health, consistent with epidemiologic health literature examining SES and BMI among youth in the U.S. and Canada (Janssen, et al., 2006; Y. Wang, 2001). Regular smoking was also found to be significantly negatively associated with SES among adolescent females, with a significant difference in the median family incomes between those who reported smoking regularly versus those who did not ($p = 0.0003$). This is consistent with the literature that smoking increases with lower socioeconomic status (Laaksonen, Rahkonen, Karvonen, & Lahelma, 2005). Although our study evaluated aggregate-level SES, a study in the U.S. found that parental SES is inversely associated with adolescent smoking (Soteriades &

DiFranza, 2003). Regular alcohol consumption was not however significantly associated with SES, which could be related to the sample consisting of adolescents who may be less likely to drink regularly due to their youth, and as well SES may not be an important indicator of regular alcohol consumption among adolescent females in this study population. A study surveying 8000 people in Rotterdam, although studying adults and not adolescents, found that excessive drinking was not significantly related to SES in women, however it was in men (van Oers, Bongers, van de Goor, & Garretsen, 1999), which apart from the age of participants is consistent with the results of this study. This result is also consistent with a review conducted by the University of British Columbia regarding socioeconomic status and health behaviours among adolescents, suggesting that there is no clear association between SES and alcohol consumption among the adolescent population (Hanson & Chen, 2007).

5.2.2.3 Associations between proximal factors and non-dominant tibial SOS (Hypothesis #3)

Age was significantly positively associated with non-dominant tibial SOS which is consistent as age is one of the most important influences on bone SOS. BMI and percentage body fat were significantly negatively associated with non-dominant tibial SOS, in accordance with previous studies examining percentage body fat and bone health (Weiler, et al., 2000; Zhao, et al., 2007). However, in our study, family history of osteoporosis, regular smoking, and alcohol consumption exhibited associations with non-dominant tibial SOS that were inconsistent with the literature and biological plausibility. These variables were dichotomous variables with only two broad categories. This led to estimates that appeared to not reflect or only borderline reflect expected results due to a greater chance for measurement error and misclassification. For these reasons, these variables were not kept in the final model.

Contrary to established research, calcium intake however was not significantly associated with non-dominant tibial SOS in univariate multiply-imputed results (p = 0.381). Absence of association may have occurred due to misclassification bias from participants because calcium intake was assessed using a self-reported questionnaire, possibly leading to an over-estimation of calcium intake, considering that the average calcium intake was much higher than typically observed in this type of sample.

5.2.2.4 Multivariable regression models

Multivariable linear regression by "intelligent" model-building was utilized in order to build a parsimonious model predicting non-dominant tibial speed of sound. Due to variables with high p-values greater than 0.2 and specified variables discussed above being inconsistent with literature being excluded, the final model included age as the key covariate. In the multivariable linear regression model, accounting for clustering and adjusting for age, median family income spline covariates were non-significant (p = 0.361). In the multivariable logistic model, accounting for the clustering effect and adjusting for age, median family income spline covariates approached significance (Wald p-value = 0.181). However, median family income spline covariates were significant in univariate analysis (Wald p-value = 0.031). Since the covariate age included in multivariable analysis can be discounted as a confounder due to the adolescent age group not being associated with SES, univariate analysis is the best model for assessing the overall effect of SES. Median family income therefore is an important predictor of bone SOS. The addition of school into the multivariable model increased the R^2 by 11.4 % in Model 5 and by 11.8% in Model 6, and school appeared to override the effect of SES in Model 6 (LRT p-value = 0.0001). This demonstrates the importance of school on bone SOS and the need to evaluate the impact of school on bone health.

5.2.2.5 Investigation of school and bone health (Hypothesis #2)

School attended was found to have a significant association with non-dominant tibial SOS in both univariate and multivariable analysis. When school was entered into the adjusted model with SES, school was significantly associated with non-dominant tibial SOS. All fixed effects models (Model 2, Model 5, Model 6, and Model 8) indicated that there is a significant school effect (LRT p-value = 0.0001 for Models 2, 5 and 6, LRT p-value = 0.002 for Model 8). While SES is important, school effect is more important. Plausible explanations and mechanisms for apparent importance of school over SES on non-dominant tibial SOS can be speculated. With 6 to 8 hours each day spent at school, school-related exposures may be more important than the level of a child or adolescent's SES.

Since school appears to have an important impact upon bone health, school impact could be explained by variation in physical activity and meal programs in schools. Murphy et al, 2006, claimed that schools were considered to provide useful settings for promoting physical activity among other health behaviours in adolescent females. This was attributed to the considerable time periods spent there, and the opportunity for formal health teaching by skilled teachers, staff encouragement and communication, and access to facilities and equipment (Murphy, et al., 2006). Even if a young adolescent female is not in control of her SES, schools could improve her bone health. Schools could be targeted for public health strategy implementation and prevention programs for low bone health, by implementing changes in the school curriculums and general attitudes towards health. In 2009, after the data for this study was collected, there were still no guidelines on vending machines or foods for secondary schools in Ontario (Finkelstein, 2009). Therefore examples of changes could be replacing pop machines with milk and dairy product vending machines in all schools; increasing and improving meal programs that include dairy products, phosphates and vitamin D; and increasing physical activity programs and sports participation, possibly by implementing mandatory gym classes past the tenth grade. School curriculum could improve health education and attitudes towards health, and physical education could be taught to be enjoyable and fun instead of being considered a chore for those adolescent girls prone to lower physical activity and sedentary activity. With improved physical activity, muscle strength increases, thereby increasing balance and decreasing the risk of falls, as increased muscle strength helps to modify and manage bone health in later years. Schools may have different levels of outdoor activities which may have an impact on vitamin D intake. Other factors pertaining to school effects such as facilities and equipment for physical activity could play a role. Schools could therefore be targeted to implement changes and improve curriculums directed towards health in general and bone health in particular, optimizing PBM, preventing poor bone health and possible future osteoporosis.

One study conducted in Canada by MacKelvie and colleagues (2001) from the University of British Columbia, although evaluating changes in bone mineral content as opposed to bone speed of sound, randomly assigned 14 elementary schools to control and intervention groups. MacKelvie and colleagues found that a school-based exercise intervention augments bone mineral accrual in early pubertal girls by 1.5% to 3.1% in the femoral neck and lumbar spine compared to the control group, providing evidence that school could be an important vehicle for improving bone health in young Canadian females (Mackelvie, McKay, Khan, & Crocker, 2001). Although school is one of many influences on young people, it is one of the few that is in regular contact with almost all young people and can have a huge impact on students' health (Sanderson, 2012).

The CDC reported that school health programs can play a critical role in promoting healthy behaviours while enhancing academic performance (Fisher, 2010). However, there is a rising problem of physical inactivity among youth in general. In the U.S., in 2001, only 32% of high school students participated in daily physical education classes, compared to 42% of students in 1991 (Fisher, 2010). Also, the Centre for Disease Control reported that 79% of young people do not eat the recommended five servings of fruits and vegetables each day (Fisher, 2010). School attended may lead to different exposures such as physical education, health promotion and nutritional programs which may explain the significant association between school and bone SOS.

Providing a Canadian context to this study, in terms of the high school curriculum in Canada, physical education classes are only mandatory in Grade 9, and beyond this it is optional. According to the Canadian Population Health Initiative, in 2004, less than one in five (16%) of Canadian schools are providing daily physical education. Also, according to the Physical & Health Education Canada, in the 2005 Survey of Canadian

Schools, although almost all elementary and middle school children took at least physical activity classes per week; a significantly lower percentage of high school students took at least one physical education class per week (Physical & Health Education Canada, 2013). In fact, there has been a declining enrollment in physical education classes from Grades 9 to 12 in Ontario beyond the base requirement for graduation (Physical & Health Education Canada, 2013). However, two thirds of Canadian schools have reported that they have policies and programs in place that encourage teachers, students and parents to be involved in organizing physical activity events and services (Physical & Health Education Canada, 2013).

5.2.2.6 Associations between school and proximal factors to non-dominant tibial SOS (Hypothesis #4)

In addition to school significantly impacting the non-dominant tibial SOS of adolescent females, school was also found to be significantly associated with percentage body fat ($p = 0.0001$), calcium intake (0.0004), and trended towards a significant association with physical activity ($p = 0.061$). School attended was also found to be significantly associated with alcohol consumption ($p = 0.0001$). These findings further contribute to the growing body of evidence investigating the impact of school on health and health-related behaviours. These findings also lend insight into the pathways that lead to the impact of school on bone health and other health outcomes. With calcium intake and physical activity significantly associated with school, it could in part explain the significant association between school and non-dominant tibial SOS. With physical activity trending towards significance, and percentage body fat highly significantly associated with school attended, it is possible that differences among schools with more physical activity programs could lead to students with lower percentage body fat. As investigated by McMurray et al. (2002), a school-based intervention can reduce body fat in young adolescents (McMurray et al., 2002).

School attended however exhibited a non-significant association with smoking ($p = 0.248$) suggesting that school does not affect smoking status of participants. Other factors likely influence youth smoking. According to the CDC, 19.1% of female high school students smoked cigarettes in the previous month in 2009 in the U.S., and that factors associated with youth tobacco use encompass other risk factors such as low SES, exposure to parental smoking, low levels of academic achievement, low self-esteem, and aggressive behaviour (Centres for Disease Control and Prevention, 2012). Therefore, school likely affects bone SOS by way of differences in physical activity and calcium intake between schools more than smoking status among female adolescents.

5.3 Strengths and Limitations

5.3.1 Study Limitations

This is a cross-sectional study and therefore does not provide information on temporality and thus provides weak support for causality. However, it is reasonable to make the connection in one direction that SES can affect a child's bone SOS rather than a child's SOS determining where the child lives and its aggregate-area level SES. A prospective cohort study would have been the superior study design, possibly starting from an earlier age, thereby tracking changes in both SES and bone SOS, and changes in intermediate factors. However evidence from this study provides a starting point for future studies, and in the future possibly conducting a randomized prevention trial in randomly selected schools and prospectively investigating its effect on bone health.

External validity is reasonable due to the moderate number of the participating schools in various locations reflecting the Canadian population. Results could be generalizable to adolescent females residing in similar locations across Canada. It has been noted that approximately 85% of the Canadian population is white (Janssen, et al., 2006) and although ethnicity data were not available in this study, this sample consisted of a mostly homogenous white population based on visual scanning of names, and data that the population in the Niagara Region is predominantly white. According to the 2006 Census, the race composition of the Niagara Region was 93.7% white and 6.3% visible minorities (Niagara Region, 2011) and the City of Hamilton was 87% white and 13% visible minorities (Statistics Canada, 2011), generally similar to the Canadian composition. Ontario's total population in 2006 was composed of 77.2% who were not visible minorities and 22.8% visible minorities, however since 98.1% of the visible minority population in Ontario live in Central Metropolitan areas (Statistics Canada, 2011), apart from major metropolitan cities the results of this study may be applicable to most of the Ontario and Canadian population. The participating schools are also generally representative of Canadian high schools as the schools were selected from both Catholic and Public School Boards. They were also in both urban and non-urban/slightly rural areas, and therefore are representative of Canadian high schools in population centres of urban and slightly rural areas. According to the 2006 Canadian Census data, 80% of the population resided in urban areas while 20% resided in rural areas (Statistics

Canada, 2011). The St. Catharines-Niagara Region was considered large urban as was the City of Hamilton by the 2011 Statistics Canada Census by population size. By population density, the Niagara Region is classified as slightly rural with 230.5 people per km^2. The City of Hamilton is classified as urban with over 400 people per km^2. All high schools except one were located in a municipality that had a population density higher than 347 people/km^2 therefore five out of the six schools were generally located in urban areas.

The use of aggregate area-based census derived SES measures versus individual data could be viewed as a limitation or strength. In the absence of quality individual-level SES data, aggregate level SES data presented a suitable alternative. However, aggregate-area level measures provide less variability and more opportunity for non-differential misclassification of exposure compared to individual-level measures, and may underestimate true effects (Janssen, et al., 2006).

There is a potential for non-differential misclassification bias of SES as described above, which biases the effect estimate towards the null. Non-differential misclassification occurs when subjects are misclassified due to inaccuracies in methods of data collection or acquisition (Gordis, 2009). Misclassification could have occurred when address data lying on the border lines of the geographical DA unit utilized could have been classified into a DA misrepresenting their SES level. It is non-differential misclassification due to the potential for residential addresses to be misclassified in either lower or higher dissemination areas, with the probability of being misclassified not differing across study participants, therefore biasing the estimate towards the null.

Misclassification bias was minimized as much as possible by the use of the dissemination area, which is the smallest census geographical unit utilized by Statistics Canada, versus larger census areas such as the census tract. Additionally, the use of aggregate-level SES is a form of misclassification as it may not truly reflect the individual SES and captures only part of the whole SES picture.

Another potential for misclassification bias was the use of self-reported data. Self-reported data for calcium intake and physical activity may have resulted in overestimations of calcium intake and physical activity and may have had an impact on the results. Moore and colleagues reported that the Rapid Assessment Method questionnaire evaluating calcium intake used in the BONES questionnaire tends to overestimate calcium intake compared to a 24-hour recall method in both child and adolescent males (Moore, Braid, Falk, & Klentrou, 2007). As well, the use of the term "regular" for the variables regular smoking and regular alcohol consumption was not defined in the questionnaire, possibly leading to misclassification of the responses.

Participation rates could have created a selection bias. Participation rates per class were unavailable however there was potentially 95% participation per class as was reported by investigators involved. Those who did not participate out of their physical education classes may have been from lower SES, since those who volunteer often come from a higher SES background. However data were not available to assess this. Those who participated may also have been healthier. The impact of volunteer bias could result in a sample unrepresentative of the population under investigation. This could have resulted in a distorted effect estimate, and likely biasing the estimate towards the null.

Also, some schools had more students participate compared to others. This makes it difficult to generalize that the findings can be extrapolated to the school level, and should be heeded with caution. In future studies, full participation from all schools should be encouraged.

The sample size in this study is adequate although a greater sample size would have increased the power of the study. For random effects multi-level analysis, ideally over 20 schools are needed in order to conduct analyses. Associations may have been attenuated due to a lack of variation in the values of bone health. The distribution of the values of the dependent variable non-dominant tibial SOS approximated a normal distribution however slightly negative-skewed since the median (3835 m/s) was greater than the mean (3822.5 m/s). Via a histogram graph and a statistical summary of the distribution of the data, the distribution of the data was also observed to be slightly negative-skewed (Skewness = -0.6). However, residuals were tested for normality in a normal quantile plot and appeared not to violate the assumption of normality.

5.3.2 Study strengths and merits:

Schools were randomly selected thereby minimizing selection bias. Schools were selected from the Niagara Catholic District School Board, and the Hamilton-Wentworth District School Board. Out of 26 schools, 6 schools were randomly sampled. However, a larger selection of schools would have improved the representation of Southern Ontario.

Aggregate level SES data presented a suitable alternative, in the absence of quality individual-level SES data. Recall and reporting bias by parents reporting their own income was curtailed with the use of aggregate-area level measures of SES. As well, as reported by the Socioeconomic Atlas, 2006, by the Ministry of Health and Long-term Care, “Research in the past decade indicate that analysis of individuals alone may not adequately capture factors associated with health status, and aggregate measures are a basic tool of population health research” (Dall, 2006).

Measurement error and inconsistencies were minimized for the measurement of bone SOS due to the use of two QUS technicians. The intra-class correlation coefficient (ICC) between the two investigators’ measurements for the four skeletal sites ranged from 0.81-0.88. However, there appeared to be measurement errors of certain proximal exposure variables that were categorical variables such as alcohol consumption, regular smoking, and family history of osteoporosis. As mentioned earlier, these variables were dichotomous variables with only two broad categories to choose from, yes versus no, leading to a greater potential for misclassification, measurement error and estimates that appeared to not reflect or only borderline reflect expected results. These variables were not kept in the final model. For future analyses, due to the “odd” and spurious nature of the findings pertaining to the variables that were dichotomous, in order to have a more robust analysis, a clear definition of the terms in the questions such as the term “regular” may minimize misclassification problems.

Anthropometric measurements such as height and weight for BMI calculations and were measured by trained researchers, and body mass (kg), lean mass and relative body fat (% body fat) were measured using the InBody520 BIA machine (Biospace Inc., Beverly Hills, USA) and thereby were not self-reported thereby minimizing misclassification bias. The BIA machine, which is based upon the principle that the electrical conductivity of fat-free tissue mass (or body water) is greater than that of fat, is a quick and easy method of assessing body composition and predicting body fat (Lukaski, Johnson, Bolonchuk, & Lykken, 1985). Some issues may arise however with participants of abnormal body composition such as those with cancer or renal diseases (Lukaski, et al., 1985), possibly resulting in errors in accuracy, however this was likely not an issue with our study.

Restricted cubic splines used to describe non-linear associations between variables was a strength as it allowed for the correct analysis and interpretation of the data as opposed to no transformation of the data.

Handling missing data by utilizing the multiple imputation method in STATA 12 also increased the validity of the study. Having a value for calcium intake for each participant and using these imputed values facilitated the goal to achieve valid statistical inference (Rubin, 1996).

Although this study was provided with many aggregate area-based census-derived estimators of SES variables, based on preliminary analysis and reasoning, in order to avoid collinearity of exposure variables and multiple comparisons, only a few SES variable were analyzed. Also, as stated by Winkleby et al., *"using multiple or composite measures may not significantly explain more about a population than would a single, well-chosen parameter"* (Winkleby, et al., 1992). Median family income and median household income were highly correlated, therefore median family income was chosen as the SES variable investigated in this study, based on the reasoning that adolescent females reside with their families.

Internal validity is moderate for this study. Some potential confounders were not reliably measured in this study, and some not measured at all, such as race. Other variables fell in the causal pathway. However, a more parsimonious model facilitated revealing an association between SES and bone SOS. The use of multi-level modeling and accounting for the clustering effect improved internal validity greatly, decreasing the chances for Type I errors. As well, as mentioned previously, selection bias was minimized by the random sampling of schools, and experimenter bias was minimized by the use of two research technicians, improving internal validity.

5.4 Public Health Relevance

This study is important as it is the first Canadian investigation that examined and provided evidence of SES as an important predictor of bone SOS among adolescent females in Canada, as well as evidence of school attended as an independent predictor of bone health among this Canadian female adolescent sample. Public health implications are that this study provides a starting point for further elucidation of the school characteristics behind the significant impact of school on bone SOS. Once these factors and mechanism are elucidated, measures can be taken including considering the implementation of intervention and prevention programs in schools targeted towards building healthier bones. The benefits from an investment into the bone health of children and adolescents likely outweighs the financial cost of intervention strategies, by optimizing a person's PBM, quality of life and ability to work. Prevention can potentially prevent a future burden on the health care system, by preventing osteoporosis and many other chronic diseases. Targeting high schools could serve as a prevention strategy for those young females who come from low SES backgrounds, since schools appear to play an important role in their bone health and PBM.

5.5 Future Research Directions

The mechanisms linking risk factors such as low SES and school to poor bone health may be elucidated from the associations found in further research. Future directions should include investigations with validation studies, further evaluation in expanded populations, and if validated, developing an understanding of mechanisms and what characteristics of school positively or negatively affect bone health. Future research investigating a larger study sample studying the association between SES and bone SOS and among other cities and schools in Canada, including a study sample representing the growing multi-ethnic population of larger Canadian cities would be beneficial. Conducting research with more schools, at least 20 schools, could make the study a random effects multi-level model. Recommendations for future studies therefore include studying a random sample of more schools along with a larger random sample of students in order to enrich the data collected of SES and bone SOS.

Thereafter a randomized prevention trial in schools could be implemented, ideally prospectively following students until later in life. Randomized prevention trials in schools would include implementing changes in school curriculums and health-related programs targeted towards healthier bones, such as replacing soda machines with dairy and milk product vending machines (which has begun in Ontario), and implementing mandatory physical education classes beyond Grade 9. Students would then be prospectively followed, allowing for the monitoring of multiple health end points including their bone health by bone SOS and calculating the incidence of poor bone health and osteoporosis, as well as monitoring for obesity and other adverse outcomes.

5.6 Conclusions

Results from this study provided evidence that SES and school are significantly associated with bone health, particularly bone SOS, among Canadian female adolescents. In univariate analysis accounting for the clustering of participants in schools, median family income (SES) was significantly non-linearly associated with non-dominant tibial SOS. School contributed significantly to the multivariable models as demonstrated by the likelihood ratio test, and overrode the effect of SES. Median family income and percentage body fat were assessed to be non-linearly associated with non-dominant tibial SOS.

The association between SES and bone SOS was found to be consistent with literature on SES and bone health; however this study provided new evidence of the association between SES and bone health among adolescent females in a Canadian population. As well this study provided new information about the possible importance of school on bone health among Canadian adolescents. This study has important implications for future research such that it has provided impetus to further investigate and identify what school characteristics mediate bone health. Once identified, school may be harnessed and utilized as a vehicle towards preventing future risk of inadequate peak bone mass. This study may facilitate improving bone health outcomes and optimizing peak bone mass in Canadian adolescent females, thereby reducing the prevalence of osteoporosis in this population, and the impact it has on quality of life, mortality and the Canadian health care system.

REFERENCES:

Akarirmak, U., Eryavuz, M, Onel, D, Rezvani, T, Karacan, I & Hatemi, H. (1996). Comparison of bone mass in primary school children at different socioeconomic levels. *Journal of Bone and Mineral Metabolism 14*(4), 229-232.

Alver, K., Sogaard, A. J., Falch, J. A., & Meyer, H. E. (2007). The Oslo Health Study: Is bone mineral density higher in affluent areas? *Int J Equity Health, 6*, 19.

Arabi, A., Nabulsi, M., Maalouf, J., Choucair, M., Khalife, H., Vieth, R., et al. (2004). Bone mineral density by age, gender, pubertal stages, and socioeconomic status in healthy Lebanese children and adolescents. *Bone, 35*(5), 1169-1179.

Association of Public Health Epidemiologists in Ontario. (2012). 2C Population Density. from http://www.apheo.ca/index.php?pid=266

Baechle, T. R., Earle, R. W. (2008). *Essentials of Strength Training and Conditioning/National Strength and Conditioning Association* (Third ed.). Champaign, IL: Human Kinetics.

Barr, S. I. (1994). Associations of social and demographic variables with calcium intakes of high school students. *J Am Diet Assoc, 94*(3), 260-266, 269; quiz 267-268.

Blimkie, C. J., Rice, S., Webber, C. E., Martin, J., Levy, D., & Gordon, C. L. (1996). Effects of resistance training on bone mineral content and density in adolescent females. *Can J Physiol Pharmacol*, *74*(9), 1025-1033.

Bonjour, J. P., Theintz, G., Law, F., Slosman, D., & Rizzoli, R. (1994). Peak bone mass. *Osteoporos Int, 4 Suppl 1*, 7-13.

Brennan S. L, H. M. J., Wluka A. E, Nicholson G. C, Kotowicz M. A, Williams J. W, Pasco J. A. (2009). BMD in Population-Based Adult Women Is Associated With Socioeconomic Status†. [Research Article]. *Journal of Bone and Mineral Research*, *24*(5), 809-815.

Brennan, S. L., Henry, M. J., Wluka, A. E., Nicholson, G. C., Kotowicz, M. A., & Pasco, J. A. (2010). Socioeconomic status and bone mineral density in a population-based sample of men. *Bone*, *46*(4), 993-999.

Brenner, D. (2008). *The role of molecular, clinical and socioeconomic factors in the long-term survival of axillary node negative breast cancer patients.* Brock University, St. Catharines.

Canadian Population Health Initiative Council. (2008). *Reducing Gaps in Health: A Focus on Socio-Economic Status in Urban Canada*.

Cashman, K. D. (2002). Calcium intake, calcium bioavailability and bone health. *Br J Nutr, 87 Suppl 2*, S169-177.

Centres for Disease Control and Prevention. (2012). Smoking & Tobacco Use. *Saving Lives. Protecting People*, 2012, from http://www.cdc.gov/tobacco/data_statistics/fact_sheets/youth_data/tobacco_use/index.htm

Chaix, B., & Chauvin, P. (2002). [The contribution of multilevel models in contextual analysis in the field of social epidemiology: a review of literature]. *Rev Epidemiol Sante Publique, 50*(5), 489-499.

Chen, E. (2010). Digging deeper: understanding the biological mechanisms that connect low socioeconomic status to poor health. *Arch Pediatr Adolesc Med, 164*(5), 495-496.

City of Hamilton. (2011). Social Determinants of Health - City of Hamilton. 2011, from www.hamilton.ca.

Clarke, B. (2008). Normal bone anatomy and physiology. *Clin J Am Soc Nephrol, 3 Suppl 3*, S131-139.

Crandall, C. J., Merkin, S. S., Seeman, T. E., Greendale, G. A., Binkley, N., & Karlamangla, A. S. (2012). Socioeconomic status over the life-course and adult bone mineral density: the Midlife in the U.S. Study. *Bone, 51*(1), 107-113.

Cummings, S. R., Black, D. M., & Rubin, S. M. (1989). Lifetime risks of hip, Colles', or vertebral fracture and coronary heart disease among white postmenopausal women. *Arch Intern Med, 149*(11), 2445-2448.

Dall, K., Lefebvre, M., Pacey, M., Sahai, V. (2006). *Socio-Economic Indicators Atlas Hamilton Niagara Haldimand Brant LHIN*.

Darwish, H., & DeLuca, H. F. (1993). Vitamin D-regulated gene expression. *Crit Rev Eukaryot Gene Expr, 3*(2), 89-116.

Davison, K. S., Siminoski, K., Adachi, J. D., Hanley, D. A., Goltzman, D., Hodsman, A. B., et al. (2006). Bone strength: the whole is greater than the sum of its parts. *Semin Arthritis Rheum, 36*(1), 22-31.

Dominguez, L. J., Scalisi, R., & Barbagallo, M. (2010). Therapeutic options in osteoporosis. *Acta Biomed, 81 Suppl 1*, 55-65.

Dorman, J. P. (2008). The effect of clustering on statistical tests: an illustration using classroom environment data. *Educational Psychology: An International Journal of Experimental Education Psychology, 28*(5), 12.

Du, X. Q., Greenfield, H., Fraser, D. R., Ge, K. Y., Liu, Z. H., & He, W. (2002). Milk consumption and bone mineral content in Chinese adolescent girls. *Bone, 30*(3), 521-528.

Dupont, W. D. (2009). *Statistical modeling for biomedical researchers : a simple introduction to the analysis of complex data* (2nd ed.). Cambridge ; New York: Cambridge University Press.

Eurostat. (2012). Population density effects on living conditions. 2012, from http://epp.eurostat.ec.europa.eu/statistics_explained/index.php/Population_density_effects_on_living_conditions

Federico, B., Falese, L., & Capelli, G. (2009). Socio-economic inequalities in physical activity practice among Italian children and adolescents: a cross-sectional study. *Z Gesundh Wiss, 17*(6), 377-384.

Finkelstein, P. (2009). School nutrition: Are we failing Canada's kids? *Nutrition* Retrieved September 2012, 2012, from http://www.besthealthmag.ca/eat-well/nutrition/school-nutrition-are-we-failing-canadas-kids

Fisher, C., Hunt, P., Kann, L., Kolbe, L., Patterson, B., Wechsler, H. (2010). *Building a Healthier Future Through School Health Programs*: Centre for Disease Control.

Fitzmaurice, G. M. (2005). Overview of Methods for Analyzing Cluster-Correlated Data. Boston: Harvard School of Public Health.

Foldes, A. J., Arnon, E., & Popovtzer, M. M. (1996). Reduced speed of sound in tibial bone of haemodialysed patients: association with serum PTH level. *Nephrol Dial Transplant, 11*(7), 1318-1321.
Foldes, A. J., Rimon, A., Keinan, D. D., & Popovtzer, M. M. (1995). Quantitative ultrasound of the tibia: a novel approach for assessment of bone status. *Bone, 17*(4), 363-367.
Fukuda, Y., Nakamura, K., Takano, T., Nakao, H., & Imai, H. (2007). Socioeconomic status and cancer screening in Japanese males: Large inequlaity in middle-aged and urban residents. *Environ Health Prev Med, 12*(2), 90-96.
Godin, G. (2011). Commentary: The Godin-Shepard Leisure-Time Physical Activity Questionnaire. *Health & Fitness Journal of Canada, 4*(1), 5.
Godin, G., & Shephard, R. J. (1985). A simple method to assess exercise behavior in the community. *Can J Appl Sport Sci, 10*(3), 141-146.
Gordis, L. (2009). *Epidemiology* (4th ed.). Philadelphia, PA: Saunders/Elsevier.
Gortmaker, S. L., Peterson, K., Wiecha, J., Sobol, A. M., Dixit, S., Fox, M. K., et al. (1999). Reducing obesity via a school-based interdisciplinary intervention among youth: Planet Health. *Arch Pediatr Adolesc Med, 153*(4), 409-418.
Gracia-Marco, L., Ortega, F. B., Casajus, J. A., Sioen, I., Widhalm, K., Beghin, L., et al. (2012). Socioeconomic status and bone mass in Spanish adolescents. The HELENA Study. *J Adolesc Health, 50*(5), 484-490.
Greer, F. R., & Krebs, N. F. (2006). Optimizing bone health and calcium intakes of infants, children, and adolescents. *Pediatrics, 117*(2), 578-585.
Hall, S. J. (2007). *Basic biomechanics* (5th ed.). Boston, Mass.: McGraw-Hill.
Hamilton Direct Info. (2011). Hamilton Direct Info. from www.hamiltondirect.info.
Hanson, M. D., & Chen, E. (2007). Socioeconomic status and health behaviors in adolescence: a review of the literature. *J Behav Med, 30*(3), 263-285.
Harrell, F. E., Jr., Lee, K. L., & Pollock, B. G. (1988). Regression models in clinical studies: determining relationships between predictors and response. *J Natl Cancer Inst, 80*(15), 1198-1202.
Health Canada. (2011). Vitamin D and Calcium: Updated Dietary Reference Intakes. 2011, from http://www.hc-sc.gc.ca/fn-an/nutrition/vitamin/vita-d-eng.php
Heaney, R. P., Abrams, S., Dawson-Hughes, B., Looker, A., Marcus, R., Matkovic, V., et al. (2000). Peak bone mass. *Osteoporos Int, 11*(12), 985-1009.
Hejazi, N., & Mazloom, Z. (2009). Socioeconomic status, youth's eating patterns and meals consumed away from home. *Pak J Biol Sci, 12*(9), 730-733.
Hennessy, T. (2013). What is middle class? *Commentary and Fact Sheets*, 2013, from http://www.progressive-economics.ca/2011/07/20/what-is-a-middle-class-income-these-days/
Hertzler, A. A., Frary, R. B. (1994). A dietary calcium rapid assessment method (RAM). *Topics in Clinical Nutrition, 9*, 76-85.
Hoglund, D., Samuelson, G., & Mark, A. (1998). Food habits in Swedish adolescents in relation to socioeconomic conditions. *Eur J Clin Nutr, 52*(11), 784-789.
Holick, M. F. (1996). Vitamin D and bone health. *J Nutr, 126*(4 Suppl), 1159S-1164S.
Hox, J. J. (1998). Multilevel modeling: When and Why? . University of Amsterdam & Utrecht University.

Humail, S. M., Samar, S., Baig, M.S., Syed, A. (2009). Comparative Study of Bone Mineral Density in High and Low Income School Children. [Original Article]. *Journal of the Dow University of Health Sciences*, *3*(2), 72-77.

Ilich, J. Z., & Kerstetter, J. E. (2000). Nutrition in bone health revisited: a story beyond calcium. *J Am Coll Nutr, 19*(6), 715-737.

James, W. P., Nelson, M., Ralph, A., & Leather, S. (1997). Socioeconomic determinants of health. The contribution of nutrition to inequalities in health. *BMJ, 314*(7093), 1545-1549.

Janssen, I., Boyce, W. F., Simpson, K., & Pickett, W. (2006). Influence of individual- and area-level measures of socioeconomic status on obesity, unhealthy eating, and physical inactivity in Canadian adolescents. *Am J Clin Nutr, 83*(1), 139-145.

Kaufman, J. S., Cooper, R. S., & McGee, D. L. (1997). Socioeconomic status and health in blacks and whites: the problem of residual confounding and the resiliency of race. *Epidemiology, 8*(6), 621-628.

Knoke, J. D., & Barrett-Connor, E. (2003). Weight loss: a determinant of hip bone loss in older men and women. The Rancho Bernardo Study. *Am J Epidemiol, 158*(12), 1132-1138.

Kogevinas, M. (1997). *Social inequalities and cancer*. Lyon, France: International Agency for Research on Cancer.

Laaksonen, M., Rahkonen, O., Karvonen, S., & Lahelma, E. (2005). Socioeconomic status and smoking: analysing inequalities with multiple indicators. *Eur J Public Health, 15*(3), 262-269.

Lanou, A. J., Berkow, S. E., & Barnard, N. D. (2005). Calcium, dairy products, and bone health in children and young adults: a reevaluation of the evidence. *Pediatrics, 115*(3), 736-743.

Lehtonen-Veromaa, M., Mottonen, T., Nuotio, I., Irjala, K., & Viikari, J. (2002). The effect of conventional vitamin D(2) supplementation on serum 25(OH)D concentration is weak among peripubertal Finnish girls: a 3-y prospective study. *Eur J Clin Nutr, 56*(5), 431-437.

Lips, P., Bouillon, R., van Schoor, N. M., Vanderschueren, D., Verschueren, S., Kuchuk, N., et al. (2010). Reducing fracture risk with calcium and vitamin D. *Clin Endocrinol (Oxf), 73*(3), 277-285.

Little, D. G., Song, K. M., Katz, D., & Herring, J. A. (2000). Relationship of peak height velocity to other maturity indicators in idiopathic scoliosis in girls. *J Bone Joint Surg Am, 82*(5), 685-693.

Lukaski, H. C., Johnson, P. E., Bolonchuk, W. W., & Lykken, G. I. (1985). Assessment of fat-free mass using bioelectrical impedance measurements of the human body. *Am J Clin Nutr, 41*(4), 810-817.

MacKelvie, K. J., Khan, K. M., Petit, M. A., Janssen, P. A., & McKay, H. A. (2003). A school-based exercise intervention elicits substantial bone health benefits: a 2-year randomized controlled trial in girls. *Pediatrics, 112*(6 Pt 1), e447.

Mackelvie, K. J., McKay, H. A., Khan, K. M., & Crocker, P. R. (2001). A school-based exercise intervention augments bone mineral accrual in early pubertal girls. *J Pediatr, 139*(4), 501-508.

Martinez, J. M., Benach, J., Benavides, F. G., Muntaner, C., Cleries, R., Zurriaga, O., et al. (2009). Improving multilevel analyses: the integrated epidemiologic design. *Epidemiology, 20*(4), 525-532.
McLellan, L., Rissel, C., Donnelly, N., & Bauman, A. (1999). Health behaviour and the school environment in New South Wales, Australia. *Soc Sci Med, 49*(5), 611-619.
McMurray, R. G., Harrell, J. S., Bangdiwala, S. I., Bradley, C. B., Deng, S., & Levine, A. (2002). A school-based intervention can reduce body fat and blood pressure in young adolescents. *J Adolesc Health, 31*(2), 125-132.
Moore, M., Braid, S., Falk, B., & Klentrou, P. (2007). Daily calcium intake in male children and adolescents obtained from the rapid assessment method and the 24-hour recall method. *Nutr J, 6*, 24.
Moro, M., van der Meulen, M. C., Kiratli, B. J., Marcus, R., Bachrach, L. K., & Carter, D. R. (1996). Body mass is the primary determinant of midfemoral bone acquisition during adolescent growth. *Bone, 19*(5), 519-526.
Mosekilde, L., Ebbesen, E. N., Tornvig, L., & Thomsen, J. S. (2000). Trabecular bone structure and strength - remodelling and repair. *J Musculoskelet Neuronal Interact, 1*(1), 25-30.
Muller, A. (2002). Education, income inequality, and mortality: a multiple regression analysis. *BMJ, 324*(7328), 23-25.
Munoz, K. A., Krebs-Smith, S. M., Ballard-Barbash, R., & Cleveland, L. E. (1997). Food intakes of US children and adolescents compared with recommendations. *Pediatrics, 100*(3 Pt 1), 323-329.
Murphy, N. M., Ni Dhuinn, M., Browne, P. A., & Orathaille, M. M. (2006). Physical activity for bone health in inactive teenage girls: is a supervised, teacher-led program or self-led program best? *J Adolesc Health, 39*(4), 508-514.
National Institute of Arthritis and Musculoskeletal and Skin Diseases. (2011). NIH Osteoporosis and Related Bone Diseases. January 2011, from http://www.niams.nih.gov/Health_Info/Bone/default.asp
National Institute of Arthritis, M. a. S. D. (2008). Bone Health for Life. In N. I. o. Health (Ed.): United States Department of Health and Human Services.
Niagara Region. (2011). Social Determinants of Health - Niagara Region. 2011, from www.niagararegion.ca.
NIH. (2011). Dietary Supplement Fact Sheet: Vitamin D 2013, from http://ods.od.nih.gov/factsheets/VitaminD-HealthProfessional/
Njeh, C. F., Saeed, I., Grigorian, M., Kendler, D. L., Fan, B., Shepherd, J., et al. (2001). Assessment of bone status using speed of sound at multiple anatomical sites. *Ultrasound Med Biol, 27*(10), 1337-1345.
Norman, G. R. S., D.L. (2008). *Biostatistics: The Bare Essentials (Third Edition)*. Shelton, CT: People's Medical Publishing House.
Novotny, R., Daida, Y. G., Grove, J. S., Acharya, S., Vogt, T. M., & Paperny, D. (2004). Adolescent dairy consumption and physical activity associated with bone mass. *Prev Med, 39*(2), 355-360.
Omar, A., Turan, S., Bereket, A. (2006). Reference data for bone speed of sound measurement by quantitative ultrasound in healthy children. *Archives of Osteoporos 1*(1-2), 37-41.

Organisation for Economic Co-operation and Development. (2011). Society at a Glance 2011: OECD Social Indicators, *Statistics* (Vol. 2012): OECD.
Osteoporosis Canada. (2010). Osteoporosis Facts and Statistics. 2010, from www.osteoporosis.ca.
Osteoporosis Canada. (2013). Exercise for healthy bones. 2013, from http://www.osteoporosis.ca/osteoporosis-and-you/exercise-for-healthy-bones/what-kind-of-activity-is-best/
Physical & Health Education Canada. (2013). Physical Education in School. from http://www.phecanada.ca/physical-education-school
Pittsburgh, U. o. (2010).
Public Health Agency of Canada. (2012). What Makes Canadians Healthy or Unhealthy? , 2012, from http://www.phac-aspc.gc.ca/ph-sp/determinants/determinants-eng.php#income
Rey, C., Combes, C., Drouet, C., & Glimcher, M. J. (2009). Bone mineral: update on chemical composition and structure. *Osteoporos Int, 20*(6), 1013-1021.
Richmond, B. (2003). DXA scanning to diagnose osteoporosis: Do you know what the results mean? *Cleveland Clinic Journal of Medicine, 70*(4), 8.
Rizzoli, R., Bonjour, J. P., & Ferrari, S. L. (2001). Osteoporosis, genetics and hormones. *J Mol Endocrinol, 26*(2), 79-94.
Roitman, J. L., & Herridge, M. (2001). *ACSM's resource manual for guidelines for exercise testing and prescription* (4th ed.). Philadelphia: Lippincott Williams & Wilkins.
Rubin, D. B. (1996). Multiple Imputation After 18+ Years. *Journal of the American Statistical Association, 91*(434).
Rubin, D. B., & Schenker, N. (1991). Multiple imputation in health-care databases: an overview and some applications. *Stat Med, 10*(4), 585-598.
Sampson, H. W. (2002). Alcohol and other factors affecting osteoporosis risk in women. *Alcohol Res Health, 26*(4), 292-298.
Sanderson, K. (2012). Health education in schools: strengths and weaknesses in relation to long-term behaviour development. *Perspect Public Health, 132*(1), 19-20.
Seeman, E. (2003). Invited Review: Pathogenesis of osteoporosis. *J Appl Physiol, 95*(5), 2142-2151.
Singh, T. P., & Evans, S. (2010). Socioeconomic position and heart rate recovery after maximal exercise in children. *Arch Pediatr Adolesc Med, 164*(5), 479-484.
Soteriades, E. S., & DiFranza, J. R. (2003). Parent's socioeconomic status, adolescents' disposable income, and adolescents' smoking status in Massachusetts. *Am J Public Health, 93*(7), 1155-1160.
StataCorp. (2009). Stata Statistical Software: Release 11 (Vol. 2012). College Station, TX: StataCorp LP.
Statistics Canada. (2011). Statistics Canada. 2011, from www.statscan.gc.ca.
Thinking with Data. (2011). Locally Weighted Scatterplot Smoothing (LOWESS). Retrieved from http://development.thinkingwithdata.com/classnotes/lowess.pdf
Thornton, L. E., Bentley, R. J., & Kavanagh, A. M. (2011). Individual and area-level socioeconomic associations with fast food purchasing. *J Epidemiol Community Health, 65*(10), 873-880.

UCLA Statistical Consulting Group. (2011). Multiple Imputation in Stata. from http://www.ats.ucla.edu/stat/stata/seminars/missing_data/mi_in_stata_pt1.htm
van Oers, J. A., Bongers, I. M., van de Goor, L. A., & Garretsen, H. F. (1999). Alcohol consumption, alcohol-related problems, problem drinking, and socioeconomic status. *Alcohol Alcohol, 34*(1), 78-88.
van Staa, T. P., Leufkens, H. G., & Cooper, C. (2002). The epidemiology of corticosteroid-induced osteoporosis: a meta-analysis. *Osteoporos Int, 13*(10), 777-787.
Wang, M. C., & Dixon, L. B. (2006). Socioeconomic influences on bone health in postmenopausal women: findings from NHANES III, 1988-1994. *Osteoporos Int, 17*(1), 91-98.
Wang, Y. (2001). Cross-national comparison of childhood obesity: the epidemic and the relationship between obesity and socioeconomic status. *Int J Epidemiol, 30*(5), 1129-1136.
Wears, R. L. (2002). Advanced statistics: statistical methods for analyzing cluster and cluster-randomized data. *Acad Emerg Med, 9*(4), 330-341.
Webb, K. L., D. (2006). *Body Basics for Bones (Second Edition)*: Birchcliff Publishing, Inc.
Weiler, H. A., Janzen, L., Green, K., Grabowski, J., Seshia, M. M., & Yuen, K. C. (2000). Percent body fat and bone mass in healthy Canadian females 10 to 19 years of age. *Bone, 27*(2), 203-207.
Weiss, M., Ben-Shlomo, A. B., Hagag, P., & Rapoport, M. (2000). Reference database for bone speed of sound measurement by a novel quantitative multi-site ultrasound device. *Osteoporos Int, 11*(8), 688-696.
West, P., Sweeting, H.,Leyland, A. (2004). School effects on pupils' health behaviours: evidence in support of the health promoting school. *Research Papers in Education, 19*(3), 29.
Willms, J. D., Fairbairn, E., Mirza, H., Tang, T. (2010). *Understanding the Early Years: Niagara Region, Ontario: A Community Research Report*.
Winkleby, M. A., Jatulis, D. E., Frank, E., & Fortmann, S. P. (1992). Socioeconomic status and health: how education, income, and occupation contribute to risk factors for cardiovascular disease. *Am J Public Health, 82*(6), 816-820.
Wolf, A. M., Gortmaker, S. L., Cheung, L., Gray, H. M., Herzog, D. B., & Colditz, G. A. (1993). Activity, inactivity, and obesity: racial, ethnic, and age differences among schoolgirls. *Am J Public Health, 83*(11), 1625-1627.
Zhao, L. J., Liu, Y. J., Liu, P. Y., Hamilton, J., Recker, R. R., & Deng, H. W. (2007). Relationship of obesity with osteoporosis. *J Clin Endocrinol Metab, 92*(5), 1640-1646.

APPENDIX 1: Brock Osteo Nutrition Exercise Study (BONES) Questionnaire

Faculty of Applied Health Sciences

Physical Education & Kinesiology

Dr. Nota Klentrou	**Dr. Kimberley Gammage**
(905) 688-5550 x 4538	(905) 688-5550 x 3772
nklentrou@brocku.ca	kgammage@brocku.ca

Socio-behavioural determinants of bone health in adolescent females

Questionnaire Package A

(Adolescent Participants)

Name: ______________________ ID: ______________________

Date of Birth: ________________ Date of Testing: ________________

DEMOGRAPHIC QUESTIONNAIRE

Your responses to this questionnaire are confidential. You may refuse to answer any of the following questions.

Father's name: ____________________________

Mother's name: ______________________

Father's Occupation: __________________________

Mother's Occupation: ________________

Father's Education Level: __________________________

Mother's Education Level: __________

Father's Address: __________________________

Mother's Address: __________________

____

____

____

____

Number of siblings: __________________________

Number of people living at home: __

Household Yearly Income:

Less than $ 20,000	$ 20,000–40,000	$ 40,000–70,000	more than $70,000
☐	☐	☐	☐

SUBJECT SCREENING AND MEDICAL HISTORY QUESTIONNAIRE

Your responses to this questionnaire are confidential. If you answer “YES” to any of the following questions, please give additional details in the space provided and discuss the matter with one of the investigators. You may refuse to answer any of the following questions.

1. Have you ever had any major joint instability or ongoing chronic pain such as in the knee, back or elbow?

   YES NO

2. Are you currently taking any medication (including aspirin) or have you taken any medication in the last two days?

   YES NO

3. Have you taken any medication in the past six months?

   YES NO

4. Is there any medical condition with which you have been diagnosed and are under the care of a physician (e.g. asthma, diabetes, anorexia)?

YES NO

5. Do you, or have you in the past, consumed any alcohol on a regular basis?

YES NO

6. Do you, or have you in the past, smoked on a regular basis?

YES NO

7. Are you, or have you in the past, engaged in any extreme diet?

YES NO

8. Do you, or have you in the past, consumed any nutritional supplements (e.g. calcium, multi-vitamin) on a regular basis?

YES NO

9. Do you, or have you in the past, taken oral contraceptives (birth control pills)?

YES NO

10. Have you ever sustained a fracture? (arm, leg)

YES NO

11. Are you, or have you in the past, had your period?

YES NO

If yes, what was your age when you first had your period? __________

12. Are your periods regular

YES NO

If yes, every how many days do you usually have your period? __________

13. Does anybody in your house smokes or has smoked on a regular basis?

YES NO

14. Is anybody in your house engaged in physical activity on a regular basis?

YES NO

If yes, who? __________ How many hours per week? __________

15. Has anybody in your family ever been diagnosed with Osteoporosis?

YES NO

If yes, who? __________

Perceived Stress Scale

INSTRUCTIONS--PLEASE READ CAREFULLY

The questions in this scale asked you about your feelings and thoughts during the last month. In each case, you will be asked to indicate *how often* you felt or thought a certain way. Although some of the questions are similar, there are differences between them and you should treat each one as a separate question. The best approach is to answer each question fairly quickly. That is, don't try to count up the number of times you felt a particular way, but rather indicate the alternative that seems like a reasonable estimate. For each question, choose from the following alternatives:

0. never
1. almost never
2. sometimes
3. fairly often
4. very often

		NEVER 0	ALMOST NEVER 1	SOMETIMES 2	FAIRLY OFTEN 3	VERY OFTEN 4
1	In the last month, how often have you been upset because of something that happened unexpectedly?					
2	In the last month, how often have you felt that you were unable to control the important things in your life?					
3	In the last month, how often have you felt nervous and “stressed”?					
4ª	In the last month, how often have you dealt successfully with irritating life hassles?					
5ª	In the last month, how often have you felt that you were effectively coping with important changes that were occurring in your life?					
6ª	In the past month, how often have you felt confident about your ability to handle your personal problems?					
7ª	In the past month, how often have you felt that things were going your way?					
8	In the past month, how often have you found that you could not cope with all the things that you had to do?					
9ª	In the last month, how often have you been able to control irritations in your life?					
10ª	In the last month, how often have you felt that you were on top of things?					
11ª	In the last month, how often have you been angered because of things that happened					

	that were outside of your control?					
12	In the last month, how often have you found yourself thinking about things that you have to accomplish?					
13[a]	In the last month, how often have you been able to control the way you spend your time?					
14	In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?					

[a] Scored in the reverse direction.

ID#
Date:

PedsQL ™

Multidimensional Fatigue Scale

Standard Version

TEEN REPORT (ages **13-18**)

DIRECTIONS

On the following page is a list of things that might be a problem for you. Please tell us **how much of a problem** each one has been for you during the **past ONE month** by circling:

0 if it is **never** a problem
1 if it is **almost never** a problem
2 if it is **sometimes** a problem
3 if it is **often** a problem
4 if it is **almost always** a problem

There are no right or wrong answers.
If you do not understand a question, please ask for help.

PedsQL (13-18) Fatigue
05/01
Questionnaire (Fatigure)

*In the past **ONE month,** how much of a **problem** has this been for you ...*

GENERAL FATIGUE *(problems with...)*	Never	Almost Never	Some-times	Often	Almost Always
1. I feel tired	0	1	2	3	4
2. I feel physically weak (not strong)	0	1	2	3	4
3. I feel too tired to do things that I like to do	0	1	2	3	4
4. I feel too tired to spend time with my friends	0	1	2	3	4
5. I have trouble finishing things	0	1	2	3	4
6. I have trouble starting things	0	1	2	3	4

SLEEP/REST FATIGUE *(problems with...)*	Never	Almost Never	Some-times	Often	Almost Always
1. I sleep a lot	0	1	2	3	4
2. It is hard for me to sleep through the night	0	1	2	3	4
3. I feel tired when I wake up in the morning	0	1	2	3	4
4. I rest a lot	0	1	2	3	4
5. I take a lot of naps	0	1	2	3	4
6. I spend a lot of time in bed	0	1	2	3	4

COGNITIVE FATIGUE *(problems with...)*	Never	Almost Never	Some-times	Often	Almost Always
1. It is hard for me to keep my attention on things	0	1	2	3	4
2. It is hard for me to remember what people tell me	0	1	2	3	4
3. It is hard for me to remember what I just heard	0	1	2	3	4
4. It is hard for me to think quickly	0	1	2	3	4
5. I have trouble remembering what I was just thinking	0	1	2	3	4
6. I have trouble remembering more than one thing at a time	0	1	2	3	4

OSTEOPOROSIS KNOWLEDGE and BELIEFS

Osteoporosis is a condition in which the bones become very brittle and weak so that they break easily. Below is a list of things that may affect a person's chance of getting osteoporosis. After each one, you are asked to circle the letters that indicate if you think the person is:

ML – MORE LIKELY TO GET OSTEOPOROSIS

LL – LESS LIKELY TO GET OSTEOPOROSIS

NT – IT HAS NOTHING TO DO WITH GETTING OSTEOPOROSIS

DK – YOU DON'T KNOW

1. Eating a diet LOW in milk products.	ML	LL	NT	DK
2. Being menopausal; female "change of life".	ML	LL	NT	DK
3. Having big bones.	ML	LL	NT	DK
4. Eating a diet high in dark-green, leafy vegetables.	ML	LL	NT	DK
5. Having a parent or grandparent who had osteoporosis.	ML	LL	NT	DK
6. Being White with fair skin.	ML	LL	NT	DK
7. Having ovaries surgically removed.	ML	LL	NT	DK
8. Taking cortisone (steroids, e.g., Prednisone).	ML	LL	NT	DK
9. Exercising regularly.	ML	LL	NT	DK

For the next group of questions, you will be asked to choose one answer from several choices. Be sure to choose only one answer. If you think there is more than one answer, choose the best answer. If you are not sure, choose "don't know".

10. Which of the following exercises is the <u>best way</u> to reduce a person's chances of getting osteoporosis?
    A. SWIMMING
    B. WALKING BRISKLY
    C. DOING KITCHEN CHORES, SUCH AS WASHING DISHES OR COOKING
    D. DON'T KNOW
11. Which of the following exercises is the <u>best way</u> to reduce a person's chance of getting osteoporosis?
    A. BICYCLING
    B. YOGA
    C. HOUSE CLEANING
    D. DON'T KNOW
12. <u>How many days a week</u> do you think a person should exercise to strengthen the bones?
    A. 1 DAY A WEEK
    B. 2 DAYS A WEEK
    C. 3 OR MORE DAYS A WEEK
    D. DON'T KNOW

13. What is the <u>least amount of time</u> a person should exercise on each occasion to strengthen the bones?
    A. LESS THAN 15 MINUTES
    B. 20 T0 30 MINUTES
    C. MORE THAN 45 MINUTES
    D. DON'T KNOW

14. Exercise makes bones strong, but it must be <u>hard enough to make breathing:</u>
    A. JUST A LITTLE FASTER
    B. MUCH FASTER, BUT TALKING IS POSSIBLE
    C. SO FAST THAT TALKING IS NOT POSSIBLE
    D. DON'T KNOW

15. Which of the following exercises is the <u>best way</u> to reduce a person's chance of getting osteoporosis?
    A. JOGGING OR RUNNING FOR EXERCISE
    B. GOLFING USING A GOLF CART
    C. GARDENING
    D. DON'T KNOW

16. Which of the following exercises is the <u>best way</u> to reduce a person's chance of getting osteoporosis?
    A. BOWLING
    B. DOING LAUNDRY
    C. AEROBIC DANCING
    D. DON'T KNOW

Calcium is one of the nutrients our body needs to keep bones strong.

17. Which of these is a good source of calcium?
    A. APPLE
    B. CHEESE
    C. CUCUMBER
    D. DON'T KNOW

18. Which of these is a good source of calcium?
    A. WATERMELON
    B. CORN
    C. CANNED SARDINES
    D. DON'T KNOW
19. Which of these is a good source of calcium?
    A. CHICKEN
    B. BROCCOLI
    C. GRAPES
    D. DON'T KNOW

20. Which of these is a good source of calcium?
    A. YOGURT
    B. STRAWBERRIES
    C. CABBAGE
    D. DON'T KNOW

21. Which of these is a good source of calcium?
    A. ICE CREAM
    B. GRAPEFRUIT
    C. RADISHES
    D. DON'T KNOW

22. Which one of the following is the recommended amount of calcium intake for an adult?
    A. 100 MG – 300 MG DAILY
    B. 400 MG – 600 MG DAILY
    C. 800 MG OR MORE DAILY

D. DON'T KNOW

23. How much milk must an adult drink to meet the recommended amount of calcium?
    A. ½ GLASS DAILY
    B. 1 GLASS DAILY
    C. 2 OR MORE GLASSES DAILY
    D. DON'T KNOW

24. Which of the following is the <u>best reason</u> for taking a calcium supplement?
    A. IF A PERSON SKIPS BREAKFAST
    B. IF A PERSON DOES NOT GET ENOUGH CALCIUM FROM DIET
    C. IF A PERSON IS OVER 45 YEARS OLD
    D. DON'T KNOW

OSTEOPOROSIS HEALTH BELIEFS

For the following questions, please indicate how strongly you agree or disagree by circling the appropriate number (1-5).

	Strongly Disagree	Disagree	Undecided	Agree	Strongly Agree
1. You feel your chances of getting osteoporosis in the future are good.	1	2	3	4	5
2. There is a good possibility that you will get osteoporosis.	1	2	3	4	5
3. Your physical health makes it more likely that you will get osteoporosis.	1	2	3	4	5
4. Your chances of getting osteoporosis are great.	1	2	3	4	5
5. Your family history makes it more likely that you will get osteoporosis.	1	2	3	4	5
6. Eating calcium rich foods requires changing your dietary habits which is difficult.	1	2	3	4	5
7. You are afraid you would not be able to always eat calcium rich foods.	1	2	3	4	5
8. Calcium rich foods do not agree with you.	1	2	3	4	5

9. Calcium rich foods are too expensive.	1	2	3	4	5
10. You dislike calcium rich foods.	1	2	3	4	5
11. You would not be so anxious about osteoporosis if you ate calcium rich foods.	1	2	3	4	5
12. Eating calcium rich foods reduces risks of broken bones.	1	2	3	4	5
13. Eating calcium rich foods helps to build bones.	1	2	3	4	5
14. Eating calcium rich foods prevents future problems from osteoporosis.	1	2	3	4	5
15. Eating calcium rich foods prevents future pain.	1	2	3	4	5
16. Exercising regularly reduces risks of broken bones.	1	2	3	4	5
17. You would not be so anxious about osteoporosis if you exercised regularly.	1	2	3	4	5
18. Exercising regularly prevents future pain.	1	2	3	4	5
19. Exercising regularly helps to build bones.	1	2	3	4	5
20. Exercising regularly prevents future problems from osteoporosis.	1	2	3	4	5

21. Exercising regularly interferes with your daily activities.	1	2	3	4	5
22. Exercising regularly can be time consuming.	1	2	3	4	5
23. Exercising regularly can be painful.	1	2	3	4	5
24. Exercising regularly would require starting a new habit which is difficult.	1	2	3	4	5
25. You are not strong enough to exercise regularly.	1	2	3	4	5
26. If you had osteoporosis, your whole life would change.	1	2	3	4	5
27. Your feelings about yourself would change if you got osteoporosis.	1	2	3	4	5
28. The thought of osteoporosis scares you.	1	2	3	4	5
29. Osteoporosis would endanger your marriage (or a significant relationship).	1	2	3	4	5
30. Having osteoporosis would make daily activities more difficult.	1	2	3	4	5
31. You frequently do things to improve your health.	1	2	3	4	5
32. You eat a well-balanced diet.	1	2	3	4	5

33. You search for new information related to your health.	1	2	3	4	5
34. You exercise regularly – at least 3 times/week.	1	2	3	4	5
35. Maintaining good health is extremely important to you.	1	2	3	4	5

OSTEOPOROSIS SELF-EFFICACY SCALE

For the following questions, indicate how confident you are with an (X) on the line.

1. Begin a new or different exercise program.

Not at all confident Very confident

2. Change your exercise habits.

Not at all confident Very confident

3. Put forth the effort required to exercise.

Not at all confident Very confident

4. Do exercises even though they are difficult.

Not at all confident Very confident

5. Maintain a regular exercise program.

Not at all confident Very confident

6. **Exercise for the appropriate length of time.**

Not at all confident | Very confident

7. **Do exercises even though they are tiring.**

Not at all confident | Very confident

8. **Stick to your exercise program.**

Not at all confident | Very confident

9. **Exercise at least three times a week.**

Not at all confident | Very confident

10. **Do the type of exercises you are supposed to do.**

Not at all confident | Very confident

11. **Begin to eat more calcium-rich foods.**

Not at all confident Very confident

12. Increase your calcium intake.

Not at all confident Very confident

13. Consume adequate amounts of calcium-rich foods.

Not at all confident Very confident

14. Eat calcium-rich foods on a regular basis.

Not at all confident Very confident

15. Change your diet to include more calcium-rich foods.

Not at all confident Very confident

16. Eat calcium-rich foods as often as you are supposed to.

Not at all confident Very confident

17. Select appropriate foods to increase your calcium intake.

Not at all confident Very confident

18. Stick to a diet which gives an adequate amount of calcium.

Not at all confident Very confident

19. Obtain foods that give an adequate amount of calcium.

Not at all confident Very confident

20. Remember to eat calcium-rich foods.

Not at all confident Very confident

21. Take calcium supplements if you don't get enough calcium from your diet.

Not at all confident Very confident

DEBQ

Please respond to the following questions by circling the appropriate number according to the following scale:

Never = 1

Seldom = 2

Sometimes = 3

Often = 4

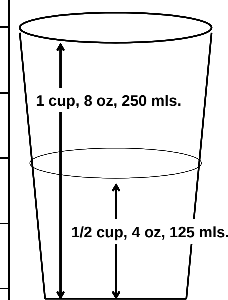
Very Often = 5

1. If you have put on weight, do you eat less than you usually do?	1	2	3	4	5
2. Do you try to eat less at meal times than you would like to eat?	1	2	3	4	5
3. How often do you refuse food or drink offered because you are concerned about your weight?	1	2	3	4	5
4. Do you watch exactly what you eat?	1	2	3	4	5
5. Do you deliberately eat foods that are slimming?	1	2	3	4	5
6. When you have eaten too much, do you eat less than usual the following day?	1	2	3	4	5
7. Do you deliberately eat less in order not to become heavier?	1	2	3	4	5
8. How often do you try not to eat between meals because you are watching your weight?	1	2	3	4	5
9. How often in the evenings do you try not to eat because you are watching your weight?	1	2	3	4	5
10. Do you take into account your weight with what you eat?	1	2	3	4	5

Rapid Assessment Method for Daily Calcium Intake

Record the number of servings you ate on *ONE typical day* in the previous 7 days.
(Use the pictures to estimate serving sizes)

MILK -YOGURT-CHEESE	# SERVINGS DAILY
Cheese, 1oz or 6 tbsp.	
Cottage cheese, ½ cup	
Custard, pudding, or cream pie, ½ cup	
Ice cream, frozen yogurt, or milk shake, 1 cup	
Milk or cocoa, 1 cup	
Soy milk, 1 cup	
Yogurt, 1 cup	
Cream soups/sauce, 1 cup	
Macaroni and cheese, 1 cup	
Pizza, 1/8 of 15" (8 slice pizza)	
Quiche, 1/8 of 8"	
FRUITS and VEGETABLES	**# SERVINGS DAILY**
Broccoli or cooked greens (beet/turnip greens, kale, collards, spinach), ½ cup	
Other vegetables, ½ cup	
Orange juice, 1 cup (enriched with calcium)	
Fruits, ½ cup or 1 small	
MEAL REPLACEMENT PRODUCTS	**# SERVINGS DAILY**
Slim fast, 1 can	
Jenny Craig bar, 1 bar	
Other:_________________________	



- **Fist = 1 cup or 1 medium whole fruit**
- **Thumb (tip to base) = 1 oz. of meat or cheese**
- **Thumb tip (tip to 1st joint) = tbsp.**
- **Index finger (1st to 2nd joint) = 1"**
- **Palm (minus fingers = 3 oz. of meat, fish, or poultry**

BREADS-CEREALS-RICE-PASTA	# SERVINGS DAILY
Bread, 1 slice	
Bread, 1 slice (enriched with calcium)	
Cereal, 1 oz	
Cereal, 1 oz (enriched with calcium)	
2” biscuit/roll	
6” corn tortilla	
3” muffin, cornbread, or doughnut	
Rice, noodles, or pasta, 1 cup	
Pancake, waffle, or French toast, 1 serving	
FAT-SUGAR-ALCOHOL	**# SERVINGS DAILY**
Cake, 1/16 of 9”	
Beer, 12oz	
Colas, 12oz	
Chocolate, 1oz	
MEAT-FISH-POULTRY-DRY BEANS-NUTS	**# SERVINGS DAILY**
Dry beans, cooked (navy, pinto, kidney), 1 cup	
Meat, fish, poultry, 3 oz	
Peanuts, ½ cup	
Almonds, ½ cup	
1 egg	
Salmon (with bones), 3oz	
Sardine (with bones), 3 oz	
3oz shrimp	
7 to 9 oysters	
Tofu, 2 ½”x 2 ½”x 1”	

GODIN-SHEPHARD LEISURE-TIME EXERCISE QUESTIONNAIRE

1. Considering a **7-day period** (a week), how many times on the average do you do the following kinds of exercise for **more than 15 minutes** during your **free-time** (write on each line the appropriate number)?

Times Per Week

(a) STRENUOUS EXERCISE

(HEART BEATS RAPIDLY) __________

(i.e. running, jogging, hockey, football, soccer, squash, basketball,

cross country skiing, judo, roller skating, vigorous swimming,

vigorous long distance bicycling)

(b) MODERATE EXERCISE

(NOT EXHAUSTING) __________

(i.e. fast walking, baseball, tennis, easy bicycling, volleyball,

badminton, easy swimming, alpine skiing, popular and folk dancing)

(c) MILD EXERCISE

(MINIMAL EFFORT) __________

(i.e. yoga, archery, fishing from river bank, bowling, horseshoes,

golf, snow-mobiling, easy walking)

2. Considering a **7-day period** (a week), during your leisure-time, how often do you engage in any regular activity long enough to work up a sweat (heart beats rapidly)?

1. OFTEN	**2. SOMETIMES**	**3. NEVER/RARELY**
☐	☐	☐

Physical Activity Questionnaire (High School)

We are trying to find out about your level of physical activity from ***the last 7 days*** (in the last week). These include sports or dance that make you sweat or make your legs feel tired, or games that make you breathe hard, like tag, skipping, running, climbing, and others.

Remember:

1. There are no right and wrong answers — this is not a test.
2. Please answer all the questions as honestly and accurately as you can — this is very important.

1. Physical activity in your spare time: Have you done any of the following activities in the past 7 days (last week)? If yes, how many times? (Mark only one circle per row.)

	No	1-2	3-4	5-6	7 times or more
Skipping	❍	❍	❍	❍	❍
Rowing/Canoeing	❍	❍	❍	❍	❍
In-line skating	❍	❍	❍	❍	❍
Tag	❍	❍	❍	❍	❍
Walking for exercise	❍	❍	❍	❍	❍
Bicycling	❍	❍	❍	❍	❍
Jogging or running	❍	❍	❍	❍	❍
Aerobics	❍	❍	❍	❍	❍
Swimming	❍	❍	❍	❍	❍
Baseball, softball	❍	❍	❍	❍	❍
Dance	❍	❍	❍	❍	❍
Football	❍	❍	❍	❍	❍
Badminton	❍	❍	❍	❍	❍
Skateboarding	❍	❍	❍	❍	❍

Soccer	❍	❍	❍	❍	❍
Street hockey	❍	❍	❍	❍	❍
Volleyball	❍	❍	❍	❍	❍
Floor hockey	❍	❍	❍	❍	❍
Basketball	❍	❍	❍	❍	❍
Ice skating	❍	❍	❍	❍	❍
Cross-country skiing	❍	❍	❍	❍	❍
Ice hockey/ringette	❍	❍	❍	❍	❍
Other:					
____________________	❍	❍	❍	❍	❍
____________________	❍	❍	❍	❍	❍

2. In the last 7 days, during your physical education (PE) classes, how often were you very active (playing hard, running, jumping, throwing)? (Check one only.)

I don't do PE .... ❍
Hardly ever .... ❍
Sometimes .... ❍
Quite often .... ❍
Always .... ❍

3. In the last 7 days, what did you normally do *at lunch* (besides eating lunch)? (Check one only.)

Sat down (talking, reading, doing schoolwork............❍
Stood around or walked around ....❍
Ran or played a little bit ....❍
Ran around and played quite a bit .... ❍
Ran and played hard most of the time .... ❍

4. In the last 7 days, on how many days *right after school*, did you do sports, dance, or play games in which you were very active? (Check one only.)

None .... ❍
1 time last week .... ❍
2 or 3 times last week .... ❍

4 times last week .... ❍
5 times last week .... ❍

5. In the last 7 days, on how many *evenings* did you do sports, dance, or play games in which you were very active? (Check one only.)

None .... ❍
1 time last week .... ❍
2 or 3 times last week .... ❍
4 or 5 last week .... ❍
6 or 7 times last week .... ❍

6. *On the last weekend*, how many times did you do sports, dance, or play games in which you were very active? (Check one only.)

None .... ❍
1 time .... ❍
2 — 3 times .... ❍
4 — 5 times .... ❍
6 or more times .... ❍

7. Which *one* of the following describes you best for the last 7 days? Read *all five* statements before deciding on the *one* answer that describes you.

F. All or most of my free time was spent doing things that involve little physical effort .... ❍

G. I sometimes (1 — 2 times last week) did physical things in my free time (e.g. played sports, went running, swimming, bike riding, did aerobics) .... ❍

H. I often (3 — 4 times last week) did physical things in my free time .... ❍

I. I quite often (5 — 6 times last week) did physical things in my free time ....❍

J. I very often (7 or more times last week) did physical things in my free time ....❍

8. Mark how often you did physical activity (like playing sports, games, doing dance, or any other physical activity) for each day last week.

	None	Little bit	Medium	Often	Very often
Monday	❍	❍	❍	❍	❍
Tuesday	❍	❍	❍	❍	❍
Wednesday	❍	❍	❍	❍	❍
Thursday	❍	❍	❍	❍	❍
Friday	❍	❍	❍	❍	❍
Saturday	❍	❍	❍	❍	❍
Sunday	❍	❍	❍	❍	❍

9. Were you sick last week, or did anything prevent you from doing your normal physical activities? (Check one.)

Yes .... ❍
No ....❍

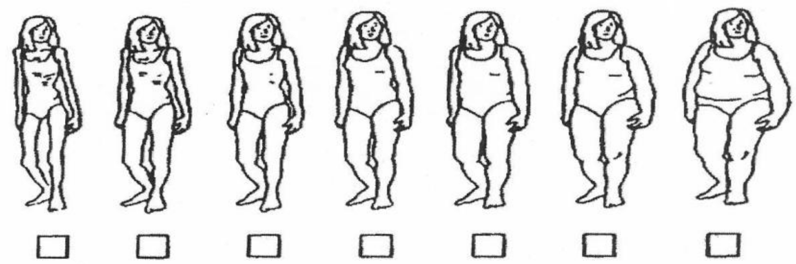
If Yes, what prevented you? ___________________________________

Child

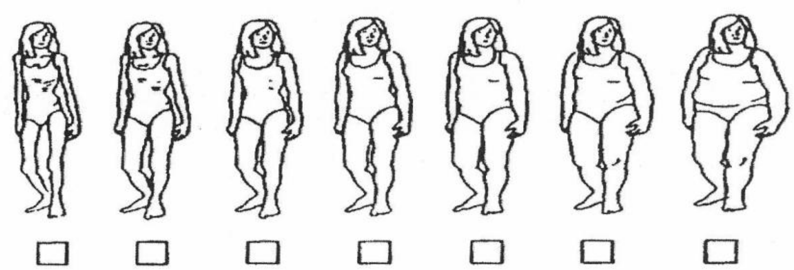
Girls Body Image Scale

Answer the questions by placing an x in the box
of the picture you choose.

Which girl do you look most like?



Which girl would you like to look like most?



APPENDIX 2: Research Ethics Board Approval for Secondary Use of Data

BREB File # 11-014

Brock University Research Ethics Board (REB) JUL 20 2011

Request for Ethics Clearance for Research Based ***Secondary Use of Data***

Researchers are encouraged to review Section 3 (Privacy and Confidentiality), Clause C (Secondary Use of Data) of the Tri-Council Policy Statement (TCPS) as well as the Brock Research Ethics Board Standard Operating Procedure relating to Secondary Use of Data prior to embarking on any secondary research. **(*Note: If documents used are all publicly available, such as newspapers, literary reviews, public policies, etc., you will not need the Research Ethic Board's approval, nor the consent of participants.)**

If you have questions about or require assistance with the completion of this form, please contact the Research Ethics Office at (905) 688-5550 ext. 3035, or reb@brocku.ca. Once complete, please return this form with all accompanying material to **MacKenzie Chown D250A**.

SECTION A – ORIGINAL INFORMATION
(Information regarding the approved protocol from which the data was first collected)

1. **Title of the Research Project:** Socio-behavioural determinants of bone health in adolescent females

2. **Principal Investigator:** Kimberley Gammage **File Number:** REB-351-06

Department: PEKN **Email:** kgammage@brocku.ca

3. **Faculty Supervisor:** **Department:**

Email: **Phone Ext.:** 3772

4. **Original Approval Date:** August 10, 2007 **Anticipated Closing Date:**

SECTION B– NEW PROTOCOL INFORMATION

	Name	Rank (e.g. faculty, student, visiting professor)	Dept./Address	Phone No.	E-Mail
Principal Investigator	Martin Tammemagi	Professor	CHSC	905-688-5550 ext.5169	martin.tammemagi@brocku.ca
Co-Investigator	Sabrina Imam	M.Sc. student	Applied Health Sciences	905-964-9224	si09gq@brocku.ca
Co-Investigator	Nota Klentrou	Professor	Kinesiology	905-688-5559 ext 4538	nklentrou@brocku.ca
Co-Investigator	Kimberley Gammage	Associate Professor	Kinesiology	905-688-5559 ext 3772	kgammage@brocku.ca

5. **Title of Project:** Investigation of the relationship between socio-economic status, schools and bone properties among adolescent females in Southern Ontario

6. **Anticipated Closing Date:** December 31, 2015

7. **Level of the Project:**

[ ] Undergraduate [ ] Masters Thesis/Project [ ] Ph.D.
[ ] Post Doctorate [X] Faculty Research [ ] Administration
[ ] Course Assignment (specify) [ ] Other (specify)

8. **Funding Status:**

Is this project currently funded? [ ] **Yes** [X] **No**

If yes, please provide the following:
Funding Sponsor:
Title of the Grant:
Period of Funding:

If no, is funding being sought? [ ] **Yes** [X] **No**
Funding Sponsor: Period of Funding:

If you are currently seeking or receiving funding, and have not yet submitted a copy of the application to the ORS, please attach a copy to this request.

9. **Contact with Other Universities:**

Has this application been submitted to another institution's Research Ethics Board? [ ] **Yes** [X] **No**

If yes, please provide the name of the Principal Investigator, Institution, date of ethics review, and decision. Attach a copy of the ethics clearance certificate, if applicable.

10. **Summary of Proposed Research:**

In lay language (100-250 words) briefly describe the purpose (objectives) and rationale of the proposed project and include and hypothesis(es)/research questions to be examined.

Osteoporosis is a condition that is characterized by low bone mass and increased susceptibility to fractures affecting nearly 2 million Canadians. It is four times more prevalent in women than in men (Osteoporosis Canada, 2011). Adequate peak bone mass must be attained in order to defend against osteoporosis later in life. Since peak bone mass is attained by late adolescence, it is crucial that research into female bone health during adolescence be conducted. Socio-economic status (SES) may be a risk factor for low peak bone mass in adolescent females, acting upon intermediate risk factors such as calcium intake, physical activity, smoking and alcohol consumption. Limited literature has examined the association between SES as a risk factor, schools and bone health among adolescent females. The proposed project aims to develop an understanding of the association between SES, schools and bone health in adolescent females in Niagara as measured by bone speed of sound (SOS). Aggregate area-based census-derived estimators of SES will be obtained by matching residential addresses previously obtained by questionnaires to aggregate level SES indices at the census area level from Statistics Canada 2006 Census data. The link to the census data will be made using subjects' addresses and study identifier numbers, and will be stripped of other study variables. We hypothesize that there is an association between SES and bone health, through mechanisms of SES acting upon intermediate risk factors for low peak bone mass.

11. **Research Participants:**

Describe the number of participants and demographics applicable to this research.

Five hundred and fifty four adolescent females in grades 9, 10 and 11 from six high schools in the Niagara and Hamilton-Wentworth School Boards will be examined.

Describe the conditions in which the data was collected initially and the reasons why it was collected.

Once selected schools were identified, the research team scheduled an information visit to each school during which they: (1) discussed the requirements of the study with teachers and participants, and (2) distributed the information letter and informed consent to be signed by the parents. Parents were asked to return a questionnaire on: parent education and household income, family history of osteoporosis, family physical activity and leisure, and family/parental smoking. Upon return of the informed consent letters, the research team then scheduled the first in school assessment visit during which participants completed the written questionnaire packet, which was composed of several questionnaires.

Questionnaires: (Each requires 10-15 min to fill out)

Subject Screening and Medical history questionnaire (Appendix A). This general questionnaire was used to assess menstrual history, smoking history, alcohol and caffeine consumption. A similar questionnaire was already approved by the Ethics Committee (file #04-284-Falk).

Perceived Stress level (Appendix B) was assessed using a stress scale, which was already evaluated for use with adolescents (Cohen et al., 1983).

The PedsQL Multidimensional Fatigue Scale. This fatigue scale (Appendix C) was previously evaluated for use with adolescents, aged (13-18 years) and had proven reliability and validity (Varni et al., 1998).

Osteoporosis Knowledge (Allinger, 1998) (Appendix D) was developed based on the self-care theory (Orem, 1995), and designed to assess women's level of osteoporosis knowledge. Initial validation of the scale on University students demonstrated acceptable content validity (0.92), a Kruder-Richardson index = 0.83, and was written at a sixth grade reading level.

Osteoporosis Health Beliefs was measured using the 35-item Osteoporosis Health Belief Scale (OHBS-Appendix E), which is based on the Health Belief Model (Kim et al., 1991). It is designed specifically to assess beliefs related to exercise behaviours and calcium intake, and consists of seven sub-scales: Seriousness, Susceptibility, Health Motivation, Calcium Benefits, Calcium Barriers, Exercise Benefits, and Exercise Barriers. Concurrent validity of the measure has been demonstrated (Stillman et al., 1986) and internal consistency for the OHBS subscales range from 0.61 for health motivation to 0.80 for susceptibility.

Osteoporosis Self-efficacy was evaluated using the Osteoporosis Self-Efficacy Scale (Appendix F), which consists of 21 items to measure self-efficacy, or confidence, for behaviours related to physical activity and calcium intake (Horan et al., 1998). Criterion-related validity was evaluated (Baecke et al., 1982) and internal consistency was >0.90.

During the second assessment in school visit, the participants underwent a Quantitative Ultrasound (QUS) test and was asked to complete nutritional and physical activity questionnaires:

Dietary Restraint was assessed using the Three-factor Eating Questionnaire (Stunkard & Messick, 1985) (Appendix G). The cognitive dietary restraint subscale was used to assess cognitive dietary restraint. This scale has shown adequate validity and reliability (Stunkard & Messick, 1985) and has been used successfully with adolescent populations (Williams et al., 1996).

Dietary Calcium Intake was estimated using the youth and adolescent food frequency questionnaire (YAQ-Appendix H) (Rockett et al. 1997). This instrument has been developed in a multi-ethnic sample of US children aged 9 to 18 years. Pearson correlation coefficients for reproducibility for nutrients ranged from 0.26 for protein and iron to 0.58 for calcium, and for foods it ranged from 0.39 to 0.57 (Rockett et al. 1995). This questionnaire was previously approved by the Brock University Ethics Committee (file #04-284-Falk).

Physical activity questionnaires: (Godin & Shephard 1985 and Fels Physical Activity Questionnaire – Treuth et al. 2005) (Appendix I). These questionnaires are designed to assess physical activity and have demonstrated adequate validity and reliability in adolescent populations (Sallis et al., 1993). Both questionnaires were previously approved by the Ethics Committee (file # 04-284-Falk).

Bone strength was determined from the speed of sound (SOS) measured by QUS (Sunlight Omnisense™ 7000S, Sunlight Medical, Ltd., Israel) as previously described (Falk et al. 2004). This ultrasound system consists of a main unit and a hand-held probe that measures the SOS (m/s) of the mid-shaft tibia and the distal 1/3 of the radius. For a detailed description of the device and technique, see Njeh et al. (1999). QUS fits World Health Organization criteria for Osteoporosis diagnosis and has been shown to be sensitive enough to detect changes in tibial QUS scores among pre-pubertal boys over an 8-month period (Falk et al. 2004). In vitro studies suggest that QUS parameters may measure previously unquantified properties of bone fragility (Gluer et al. 1993). Previous studies using calcaneal ultrasound have also demonstrated that QUS can predict vertebral and hip fracture (Bauer et al.1995; Njeh et al. 1997; Porter et al. 1990). This procedure was previously approved by the Ethics Committee (file #04-284-Falk).

Anthropometric measures, including body mass & height, segmental/limb lengths and circumferences, was measured using standard methods (Durnin and Whomersley 1974). This procedure was previously approved by the Ethics Committee (file #04-284-Falk).

Body fat composition using bio-electrical impedance analysis (BIA): The BIA device creates a mild electrical current (50kHz, 800μA) that passes from electrodes situated on the dorsal part of the hand to electrodes on the dorsal part of the foot. This current is very low and one cannot feel it. There is no discomfort is associated with this measurement (files # Gurd-00-082, Cieslak 01-010).

Please attach a copy of the consent form from which the data was originally collected. Evaluate and comment on the degree of expectations the individuals who provided the information had regarding their data being kept confidential, and unused for other purposes.

Consent form is attached. Confidentiality was explained orally and in the consent form. Participants were informed that the data may be used for secondary analysis by the investigators. Individuals most likely expected their data to be kept confidential and anonymous by the use of participant ID number only which remains the case for this study.

12. Free and Informed Consent:

Indicate from which organization or institution the data will be obtained (Please attach the letter of approval from that organization concerning the use of the data they collected). Brock Osteo Nutrition and Exercise Study (BONES), Brock University

Indicate, if applicable, how you will obtain free and informed consent of research participants ***(It is possible, in some cases, that the consent of participants is necessary to obtain. This becomes necessary when data can be linked to individuals, and is critical when the possibility exists that individuals can be identified in published reports. See the Tri-Council Policy Statement, C.3., Secondary Use of Data for further information).***

Not applicable. No reports will be published in which individual level data are reported. It will not be possible to identify any individual from any report, publication or presentation resulting from the current study.

***Please submit the consent form or information sheet to be given to the research participants.**

13. **Proportionality of Harms and Benefits:**

Indicate if the methods used (previous research) risk causing harm or inconvenience to the research participants. Describe the nature of such harms or the potential consequences on any legal, physical, psychological, or social aspect associated with each procedure in the research or methods used.

The anthropometric and bone assessments involved minor bodily contact. This level of bodily contact was familiar to all subjects through their regular medical check-ups. No researcher was ever left alone with a participant. Possible benefits were that participants gained personal and general knowledge about the human body. In addition, they gained knowledge about their own bone health.

Evaluate the level of physical or emotional harm or discomfort that the research could create for the research participants (i.e. none, low, moderate, high). Indicate the measures you have taken to minimize such harms.

None. In the current study, no personal contact will be made with the study subjects, so there will be no additional burden on study subjects, beyond what was required in the original study.

Justify the potential harms by describing the anticipated benefits of the research (for general knowledge and for the research participants), and the way benefits will be maximized.

The knowledge gained from this research will help identify female adolescents who are at high risk for poor bone health and future risk of osteoporosis. This knowledge may help provide this at-risk group with interventions to overcome low bone health in their adolescence, such as information or active programs that modify smoking, dietary, and exercise behaviours.
Although our study is unlikely to help the study subjects at this time, it may help reduce the morbidity and mortality associated with osteoporosis in the future, across Canada and possibly more broadly.
To disseminate of our study findings, they will be presented in major scientific conferences and will be published in peer-reviewed scientific journals.

14. **Privacy of Research Participants:**

Specify how you will ensure the anonymity of the research participants. If anonymity is not to be guaranteed, explain how the research participants will be informed of that fact.

Personal identifiers, such as names, are removed from the study database. The subjects' addresses are in a separate file identified only by study ID number, which will enable linking of the address-derived SES data back to the main study dataset.
All reports will publish pooled results so that no individual statistics will be made public. Similarly, any maps will be drawn with grids and scales making it impossible to identify subjects' dwellings, streets or blocks.
All hard copy study records will be kept in a locked cabinet in a locked room, to which public access is restricted.
All study data on computer will be kept on limited access, password protected computers. Only study Principal and Co-Investigators will have access to the study analytic database.

15. **Confidentiality of Data:**

Specify who will have access to the data collected, where the data will be stored, how long the data will be preserved, and what particular measures will be taken to ensure its confidentiality.

Only the Principal and Co-Investigators will have access to the complete research dataset. A medical geographer, Mr. Ryan Waterhouse, from the Niagara Region Department of Public Health will have access to subjects' addresses to be able to link the addresses with census data to obtain the group level SES data.
Hard copy data is stored in a locked cabinet in a locked room in the Department of Kinesiology. Computer data will be kept on password protected computers belonging to Dr. Martin Tammemagi, Department of Community Health Sciences (Walker Complex, Academic South, room 306) and graduate student, Sabrina Imam, Applied Health Sciences.
Data will be stored in a database located in a secure password protected computer and will be preserved no longer than 5 years after the last planned analysis and publication.
Steps to ensure confidentiality have been described. In summary, direct access to the data is limited to a small group of researchers. The hard copy data and computer data will be kept in physical places or computers which are secure and protected by key or password, respectively. The analytic dataset will not contain personal identifiers.

SECTION C – SIGNATURES

Principal Investigator:

Please indicate that you have read and fully understand all ethics obligations by checking the box beside each statement.

[X] I have read Section III:8 of Brock University's Faculty Handbook pertaining to Research Ethics and agree to comply with the policies and procedures outlined therein.
[X] I will report any serious adverse events (SAE) to the Research Ethics Board (REB).

[X] Any additions or changes in research procedures after approval has been granted will be submitted to the REB.
[X] I agree to request a renewal of approval for any project continuing beyond the expected date of completion or for more than one year.
[X] I will submit a final report to the Office of Research Services once the research has been completed.
[X] I take full responsibility in ensuring that all other investigators involved in this research follow the protocol as outlined in the application.

Signature [redacted] Date: [redacted]

Faculty Supervisor(s):

Please indicate that you have read and fully understand the obligations as faculty supervisor listed below by checking the box beside each statement.

[ ] I agree to provide the proper supervision of this study to ensure that the rights and welfare of all human participants are protected.
[ ] I will ensure a request for renewal of a proposal is submitted if the study continues beyond the expected date of completion of for more than one year.
[ ] I will ensure that a final report is submitted to the Office of Research Services.
[ ] I have read and approved the application and proposal.

Signature ______________________________ Date: ______________

Signature ______________________________ Date: ______________

***Office Use Only**

This request for access to secondary use of data involving human participants has been reviewed and received ethics clearance.

____

Chair, Research Ethics Board **Date**

Brock

Faculty of Applied Health Sciences
Department of Kinesiology

Brock University
Niagara Region
500 Glenridge Avenue
St. Catharines, ON
L2S 3A1 Canada
T 905 688 5550 x4957
F 905 688 8364

brocku.ca

July 18, 2011

To: Research Ethics

Re: Request for use of data for Secondary use of Research Data

This letter is to verify that Dr. Martin Tammemagi and Sabrina Imam (MSc Candidate) have permission to use data from the BONES study (Socio-behavioural determinants of bone health in adolescent females, REB #06-351) for the study as outlined in their research ethics application. The data provided will have no unique identifying information attached to it. If you have any further questions, please feel free to contact us.

Sincerely,

[redacted]

Kimberley L. Gammage
Associate Professor
Department of Kinesiology
Brock University
kgammage@brocku.ca

Panagiota Klentrou
Professor
Department of Kinesiology
Brock University
nklentrou@brocku.ca

APPENDIX 3: Brock Osteo Nutrition Exercise Study (BONES) Invitation to Parents

Invitation Letter

Socio-behavioural determinants of bone health in adolescent females

Principal Investigators: Dr. Nota Klentrou and Dr. Kimberley Gammage, Department of Physical Education and Kinesiology, Brock University

We would like to invite your daughter to participate in the present study, which investigates socio-behavioural determinants of bone health as assessed by a new ultrasound technique, in adolescent females.

The **purpose** of this research project is to examine young females' specific socio-behavioural factors related to osteoporosis, and will evaluate lifestyle risk factors and preventative behaviours.

Tests and measurements will be completed during two visits in your school of approximately 1 hour each. Briefly, measurements include bone strength (using ultrasound), physical characteristics, and filling out several questionnaires.

Participation in this project will allow you to have personal information on your bone strength, as well as other information, such as height, weight and percent body fat.

This research is being performed only by Brock University researchers in the Applied Physiology Laboratory.

If you have any pertinent questions about your rights as a research participant, please contact the Brock University Research Ethics Officer (905 688-5550 ext 3035, reb@brocku.ca)

If you have any questions, please feel free to contact us.

Thank you

Nota Klentrou, PhD

Department of Physical Education and Kinesiology
Faculty of Applied Health Sciences
Brock University
Tel: 905-688-5550 ext 4538
email: <nota.klentrou@brocku.ca>

This study has been reviewed and received ethics clearance through Brock University's Research Ethics Board (file # 06-351)

APPENDIX 4: Brock Osteo Nutrition Exercise Study (BONES) Consent Form

CONSENT/ASSENT TO PARTICIPATE IN RESEARCH

Socio-behavioural determinants of bone health in adolescent females

You are being invited to participate in a research study being conducted by the investigators listed below. Prior to participating in this study please read this form to find out about the purpose and the tests of this study. This study is part of the Department of Physical Education and Kinesiology (PEKN) in the Faculty of Applied Health Sciences of Brock University.

INVESTIGATOR:	DEPARTMENT:	CONTACT:
Dr. Nota Klentrou	PEKN, Brock University	(905) 688-5550 ex. 4538
Dr. Kimberley Gammage	PEKN, Brock University	(905) 688-5550 ex. 3772

PURPOSE:

The purpose of this project is application will examine young females' specific socio-behavioural factors related to osteoporosis, and will evaluate lifestyle risk factors and preventative behaviours.

DESCRIPTION OF TESTING PROCEDURES:

The questionnaires and tests described below will be completed during two visits in your school of approximately 1 hour each. At the end of the study, a summary of your personal results and the summarized findings will be available, upon request. The procedures include:

1. Completion of several questionnaires, outlining your medical history, perceived stress, fatigue levels, osteoporosis knowledge, self-efficacy, health beliefs, physical activities and nutritional habits. In all questionnaires, you may chose not to answer any question without penalty. ***Parents will also be asked to return a questionnaire on: parent education and household income, family history of osteoporosis, family physical activity and leisure, and family/parental smoking. A SELF-ADDRESSED ENVELOPE WILL BE PROVIDED.***
2. Determination of your physical characteristics, including height and weight, arm and leg length and circumference. This procedure is quick and causes no discomfort.
3. Estimation of relative body fat using bioelectrical impedance analysis. The BIA device creates a mild electrical current (50kHz, 800 µA) that passes from electrodes situated on the back of your hand, through the body, to electrodes on the top of your feet. This current is very low and one cannot feel it. The measurement is quick, and no discomfort is associated with this measurement.
4. Bone strength will be determined from the speed of sound (SOS) measured by Quantitative Ultrasound (Sunlight Omnisense™ 7000S, Sunlight Medical, Ltd., Israel). This ultrasound system consists of a main unit and a hand-held probe that measures the

SOS (m/s) of the mid-shaft tibia and the distal 1/3 of the radius. The measurement quick and no discomfort is associated with this measurement.

CONFIDENTIALITY

All data collected during this study will remain confidential and will be stored in offices and on secured computers to which only the principal and co-investigators have access. You should be aware that the results of this study may be made available to scientists, through publication in a scientific journal but your name and any personal data of you will not appear in the compiling or publishing these results. Additionally, you will have access to your own data, as well as the group data when it becomes available and if you are interested.

PARTICIPATION & WITHDRAWL

You can choose whether to participate in this study or not. You may remove your data from the study if you wish. You may also refuse to answer any questions posed to you during the study and still remain as a subject in the study. The investigators reserve the right to withdraw you from the study if they believe that it is necessary.

RISKS AND BENEFITS

Participation will allow you to gain personal and general knowledge about the human body and your bone health status. Additionally, if any unusually low or high result is attained for any of the measurements, reflecting a possible health-related problem, you and/or your parents will be alerted and advised to consult your physician. All results will be provided to you and/or your parents upon request. There are no foreseeable risks in your participation in this research study.

RIGHTS OF RESEARCH PARTICIPANTS

You will receive a signed copy of this ethics form. You may withdraw your consent to participate in this study at any time, and you may also discontinue participation at any time without penalty. In signing this consent form or in participating in this study, you are not waiving any legal claims or remedies. This study has been reviewed and received clearance from the Brock University Ethics Board (file # 06-351). If you have any pertinent questions about your rights as a research participant, please contact the Brock University research Ethics Office (905-688-5550 ext. 3035, reb@brocku.ca)

INFORMATION:

Please contact Dr. Nota Klentrou at 905-688-5550 ex 4538 or Dr. Kimberley Gammage at 905-688-5550 ex 3772, if you have any questions about the study.

I HAVE READ AND UNDERSTAND THE ABOVE EXPLANATION OF THE PURPOSE AND PROCEDURES OF THE PROJECT. I HAVE ALSO RECEIVED A SIGNED COPY OF THE INFORMATION

AND CONSENT FORM. MY QUESTIONS HAVE BEEN ANSWERED TO MY SATISIFACTION AND I AGREE TO PARTICIPATE IN THIS STUDY.

SIGNATURE of PARENT/GUARDIAN DATE

PRINTED NAME OF PARTICIPANT DATE

WITNESS DATE

PRINTED NAME OF WITNESS

INVESTIGATOR

In my judgment, the participant is voluntarily and knowingly giving informed consent and possesses the legal capacity to give informed consent and participate in this research study.

SIGNATURE OF INVESTIGATOR DATE

APPENDIX 5: Brock Osteo Nutrition Exercise Study (BONES) Letter to Schools

Example Letter to the Principal

Name of High School

Dear Principal,

We have received permission from the **Name of School Board** to conduct our study entitled "Socio-Behavioural Determinants of Bone Health in Adolescent Females", and we have randomly selected your school to participate. This novel research program aims to examine the socio-behavioural determinants of optimal bone health and osteoporosis risk factors in adolescent females, and to develop, implement and evaluate potential population specific interventions sensitive to the pathways that connect socio-behavioural determinants to this chronic health program. The first stage of this research will examine young females' specific socio-behavioural factors related to osteoporosis, and will evaluate lifestyle risk factors and preventative behaviours, such as menstrual history, smoking, caffeine intake, and dietary and exercise habits.

Participation will allow students to gain personal and general knowledge about the human body and their bone health status. Additionally, if any unusually low or high result is attained for any of the measurements, reflecting a possible health-related problem, the student and/or parents will be alerted and advised to consult their physician. Personal data will be kept confidential and will be available to the students and/or parents only upon request. There are no foreseeable risks in participating in this research study.

The number of students required from **Name of High School** will be approximately 100 from grades 9, 10 and 11. The participants will be required for two visits, the first being dedicated to the in-class completion of a number of questionnaires. This will take approximately one period and preferably done during their Physical Education or Exercise Science class. The second visit will consist of anthropometric measurements including height, weight and percent body fat (using a non-invasive and touch free bioelectrical impedance device), as well as a bone ultrasound at the tibia and radius. This visit will take about twenty minutes of their Physical Education or Exercise Science class.

The research team would like to schedule a meeting with you to discuss this project in more detail and to confirm a start date. We are willing to make a presentation relating to bone health and supply additional material for the teachers and students for better incorporation of this research into the PE curriculum. Visits to the University laboratories can also be arranged upon request by teachers. Please contact the project coordinator Brianna Holmes (by email: bh03iv@brocku.ca or by phone: (905) 688-5550 ext 5623) to set up a meeting at your earliest convenience.

Sincerely,

Panagiota (Nota) Klentrou

Professor and Chair

Department of Physical Education & Kinesiology

Faculty of Applied Health Sciences

Physical Education & Kinesiology

Dr. Nota Klentrou Dr. Kimberley Gammage

(905) 688-5550 x 4538 (905) 688-5550 x 3772

nklentrou@brocku.ca kgammage@brocku.ca